APPLICATION

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TITLE: METHODS AND PROBES FOR THE DETECTION OF CANCER

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METHODS AND PROBES FOR THE DETECTION OF CANCER

Cross Reference To Related Applications

This application claims priority from U.S. Provisional Patent Application Serial No. 60/270,271, filed February 20, 2001. This application is incorporated herein by reference in its entirety.

Field of the Invention

The invention relates to methods and probes for the detection of cancer.

Background of the Invention

Lung cancer is the leading cause of death due to cancer in the United States, killing approximately 156,000 men and women each year. There are four major bronchogenic carcinoma cell types that account for over 95% of primary lung cancers: adenocarcinoma; squamous cell carcinoma; large cell carcinoma; and small cell carcinoma. These cell types occur singly or in combination. The remaining 5% of tumors are composed of several unusual tumor types.

When lung cancer develops, it tends to spread from the original cancer site to the lymph nodes, and then, either at the same time or sequentially, to other areas of the body. The most common sites for lung cancer spread (metastasis) are the brain, bones, liver, adrenal glands, and any other organ with a high rate of blood flow. It is this process of metastasis that leads to fatality in most patients.

When a cancer is first discovered by physical examination or by diagnostic tests (e.g., X-ray or high resolution imaging such as spiral CT), it is usually at least 1 cm in size. A cancer that is 1 cm in size contains at least about 1 billion cells.

Changes in chromosomal DNA have been shown to accompany the conversion of normal cells to malignant cells. Because of this, detection of specific chromosomal alterations provides a route to detecting and diagnosing lung cancer.

Summary of the Invention

The invention is based on the discovery that specific probes and probe sets can be used to detect lung cancer with high levels of sensitivity. By using the probes described herein, lung cancer can be detected with enhanced sensitivity as compared to conventional methods. Accordingly, the probes and methods of the invention facilitate the detection of lung cancer and/or allow for the detection of lung cancer at early stages. The invention includes probe sets, methods of using probes and probe sets, and methods of selecting probe sets for the detection of cancer.

In one aspect, the invention features set of chromosomal probes including any of the following combinations of two probes: (a) a 5p chromosome arm probe and a probe selected from the group consisting of a 8q24 locus specific probe, a 3q chromosome arm probe, a 20q chromosome arm probe, a 7p12 locus specific probe, a chromosome 16 enumeration probe, a chromosome 4 enumeration probe, a chromosome 12 enumeration probe, a chromosome 6 enumeration probe, and a 17q21 locus specific probe; (b) a 8q24 locus specific probe and a probe selected from the group consisting of a chromosome 17 enumeration probe, a chromosome 1 enumeration probe, and a chromosome 6 enumeration probe; (c) a 7p12 locus specific probe and a probe selected from the group consisting of a 3q chromosome arm probe and a chromosome 6 enumeration probe; (d) a 3q chromosome arm probe and a chromosome 7 enumeration probe; or (e) a chromosome 6 enumeration probe and a chromosome 7 enumeration probe.

A detection moiety can be attached to the two probes. The detection moiety can contain a fluorescent label. The two probes can optionally be coupled to different detection moieties. For example, the detection moieties can contain fluorescent labels.

In another aspect, the invention features a set of chromosomal probes including any of the following combinations of three probes: (a) a 5p15 locus specific probe, a 8q24 locus specific probe, and a probe selected from the group consisting of a 9p21 locus specific probe, a chromosome 1 enumeration probe, a chromosome 6 enumeration probe, a 7p12 locus specific probe, and a 17q21 locus specific probe; (b) a 5p15 locus specific probe, a chromosome 12 enumeration probe, and a 9p21 locus specific probe; (c) a 8q24 locus specific probe, a chromosome 17 enumeration probe, and a 9p21 locus specific probe; (d) a 8q24 locus specific probe, a chromosome 1 enumeration probe, and a 9p21

locus specific probe; or (e) a 5p15 locus specific probe, a 3q chromosome arm probe, and a chromosome 12 enumeration probe.

In another aspect, the invention features a set of chromosomal probes including any of the following combinations of four probes: (a) a 5p15 locus specific probe, a chromosome 6 enumeration probe, a 17p13 locus specific probe, and a chromosome 17 enumeration probe; (b) a 5p15 locus specific probe, a 8q24 locus specific probe, a chromosome 1 enumeration probe, and a 7p12 locus specific probe; (c) a 5p15 locus specific probe, a 8q24 locus specific probe, a 3q chromosome arm probe, and a 7p12 locus specific probe; (d) a 5p15 locus specific probe, a 8q24 locus specific probe, a 20q chromosome arm probe, and a 7p12 locus specific probe; (e) a 5p15 locus specific probe, a 8q24 locus specific probe, a 7p12 locus specific probe, and a 17q21 locus specific probe; (f) a 5p15 locus specific probe, a 8q24 locus specific probe, a chromosome 6 enumeration probe, and a 7p12 locus specific probe; (g) a 5p15 locus specific probe, a 8q24 locus specific probe, a chromosome 6 enumeration probe, and a chromosome 1 enumeration probe; (h) a 5p15 locus specific probe, a 8q24 locus specific probe, a chromosome 6 enumeration probe, and a chromosome 12 enumeration probe; (i) a 5p15 locus specific probe, a chromosome 1 enumeration probe, a chromosome 6 enumeration probe, and a chromosome 12 enumeration probe; (j) a chromosome 7 enumeration probe, a chromosome 1 enumeration probe, a chromosome 6 enumeration probe, and a chromosome 12 enumeration probe; or (k) a 5p chromosome arm probe, a chromosome 1 enumeration probe, a chromosome 6 enumeration probe, and a chromosome 7 enumeration probe.

In some embodiments of the probe sets described herein, e.g., a set containing at least two, three, or four probes, a 5p chromosome arm probe can be used in place of a 5p15 locus specific probe. In other embodiments of the probe sets described herein, a 7p chromosome arm probe can be used in place of a 7p12 locus specific probe.

In another aspect, the invention features a method of screening for lung cancer in a subject, the method including the steps of: (a) obtaining a biological sample from the subject; (b) obtaining a set of at least two different chromosomal probes, e.g., at least two, three, or four probes, from a set described herein; (c) contacting the set of probes to the biological sample under conditions sufficient to enable hybridization of probes in the

set to chromosomes in the sample, if any; and (d) detecting the hybridization pattern of the set of chromosomal probes to the biological sample to determine whether the subject has lung cancer.

The probes used in the methods described herein can be selected from the group consisting of a chromosome 1 enumeration probe, a chromosome 3 enumeration probe, a chromosome 4 enumeration probe, a chromosome 6 enumeration probe, a chromosome 7 enumeration probe, a chromosome 8 enumeration probe, a chromosome 9 enumeration probe, a chromosome 10 enumeration probe, a chromosome 11 enumeration probe, a chromosome 12 enumeration probe, a chromosome 16 enumeration probe, a chromosome 17 enumeration probe, a chromosome 18 enumeration probe, a 3p14 locus specific probe, a 3q26 locus specific probe, a 5p15 locus specific probe, a 5q31 locus specific probe, a 7p12 locus specific probe, a 8q24 locus specific probe, a 9p21 locus specific probe, a 10q23 locus specific probe, a 13q14 locus specific probe, a 17p13 locus specific probe, a 17q21 locus specific probe, a 20q13 locus specific probe, a 21q22 locus specific probe, a 3q chromosome arm probe, a 5p chromosome arm probe, a 7p chromosome arm probe, a 3p chromosome arm probe, and a 20q chromosome arm probe.

The biological sample used in the methods described herein can contain a bronchial specimen, a lung biopsy, or a sputum sample. The chromosomal probes used in the methods described herein can optionally be fluorescently labeled. The methods described herein can further include performing cytological analysis on the sample.

In another aspect, the invention features a method of screening for lung cancer in a subject, the method including the steps of: (a) obtaining a biological sample from the subject; (b) obtaining a chromosomal probe selected from the group consisting of a 5p15 locus specific probe, a chromosome 1 enumeration probe, a 7p12 locus specific probe, a 8q24 locus specific probe, and a chromosome 9 enumeration probe; (c) contacting the chromosomal probe to the biological sample under conditions sufficient to enable hybridization of the probe to chromosomes in the sample, if any; and (d) detecting the hybridization pattern of the probe to the biological sample to determine whether the subject has lung cancer.

In another aspect, the invention features a method of selecting a combination of probes for the detection of cancer, the method including the steps of: (a) providing a first

plurality of chromosomal probes; (b) determining the ability of each of the first plurality of probes to distinguish cancer specimens from normal specimens; (c) selecting those probes within the first plurality of probes that identify the cancer specimens as compared to the normal specimens to yield a second plurality of probes, wherein the second plurality of probes each identify the cancer specimens as compared to the normal specimens at a p value of less than 0.01 or a vector value of less than 0.500; (d) determining the ability of a combination of probes selected from the second plurality of probes to distinguish the cancer specimens from the normal specimens; and (e) selecting a combination of probes that identifies the cancer specimen as compared to the normal specimen with a vector value of less than 0.400.

In one embodiment, the cancer specimens are lung cancer specimens. For example, the specimens can be derived from patients diagnosed as having lung cancer. The normal specimens can be lung tissue specimens derived from patients not diagnosed as having lung cancer.

In one embodiment, step (c) of the method includes selecting those probes within the first plurality of probes that identify the cancer specimens as compared to the normal specimens to yield a second plurality of probes, wherein the second plurality of probes each identify the cancer specimens as compared to the normal specimens at a p value of less than 0.005 or 0.001 and/or a vector value of less than 0.400, 0.300, 0.200, or 0.100.

In another embodiment, step (e) of the method includes selecting a combination of probes that identifies the cancer specimen as compared to the normal specimen with a vector value of less than 0.300, 0.200, or 0.100.

In another aspect, the invention features a set of chromosomal probes including at least two different probes, wherein the set of probes is capable of detecting lung cancer with a sensitivity of at least about 60%, e.g., when tested on a population containing at least 35 lung cancer patients.

In one example, the set contains at least three different probes. In another example, the set contains at least four different probes.

In one example, the set is capable of detecting lung cancer with a sensitivity of at least about 60% at a cutoff value of about 10%. In another example, the set is capable of detecting lung cancer with a sensitivity of at least about 70% when the detection is

performed on a biological sample containing a bronchial specimen. In another example, the set is capable of detecting lung cancer with a sensitivity of at least about 80% at a cutoff value of about 20%.

The chromosomal probes contained in the sets described herein, e.g., sets of at least two, three, or four different probes, can be selected from the group consisting of a chromosome 1 enumeration probe, a chromosome 3 enumeration probe, a chromosome 4 enumeration probe, a chromosome 6 enumeration probe, a chromosome 7 enumeration probe, a chromosome 8 enumeration probe, a chromosome 9 enumeration probe, a chromosome 10 enumeration probe, a chromosome 11 enumeration probe, a chromosome 12 enumeration probe, a chromosome 16 enumeration probe, a chromosome 17 enumeration probe, a chromosome 18 enumeration probe, a 3p14 locus specific probe, a 3q26 locus specific probe, a 5p15 locus specific probe, a 5q31 locus specific probe, a 7p12 locus specific probe, a 8q24 locus specific probe, a 9p21 locus specific probe, a 10q23 locus specific probe, a 13q14 locus specific probe, a 17p13 locus specific probe, a 17q21 locus specific probe, a 20q13 locus specific probe, a 21q22 locus specific probe, a 3q chromosome arm probe, a 5p chromosome arm probe, a 7p chromosome arm probe, a 3p chromosome arm probe, and a 20q chromosome arm probe.

In another aspect, the invention features a set of chromosomal probes including at least two different probes, wherein the set is capable of detecting lung cancer with a vector value of less than 0.500, e.g., when tested on a population containing at least 35 lung cancer patients and 20 normal individuals.

In one example, the set is capable of detecting lung cancer with a vector value of less than 0.500 at a cutoff value of about 10%. In another example, the set is capable of detecting lung cancer with a vector value of less than 0.400. In another example, the set is capable of detecting lung cancer with a vector value of less than 0.400 at a cutoff value of about 15%. In another example, the set is capable of detecting lung cancer with a vector value of less than 0.300. In another example, the set is capable of detecting lung cancer with a vector value of less than 0.300 at a cutoff value of about 15%. In another example, the set is capable of detecting lung cancer with a vector value of less than 0.200. In another example, the set is capable of detecting lung cancer with a vector value of less than 0.200 at a cutoff value of about 20%.

The at least two different probes of the set can be selected from the group consisting of a chromosome 1 enumeration probe, a chromosome 3 enumeration probe, a chromosome 4 enumeration probe, a chromosome 6 enumeration probe, a chromosome 7 enumeration probe, a chromosome 8 enumeration probe, a chromosome 9 enumeration probe, a chromosome 10 enumeration probe, a chromosome 11 enumeration probe, a chromosome 12 enumeration probe, a chromosome 16 enumeration probe, a chromosome 17 enumeration probe, a chromosome 18 enumeration probe, a 3p14 locus specific probe, a 3q26 locus specific probe, a 5p15 locus specific probe, a 5q31 locus specific probe, a 7p12 locus specific probe, a 8q24 locus specific probe, a 9p21 locus specific probe, a 10q23 locus specific probe, a 13q14 locus specific probe, a 17p13 locus specific probe, a 17q21 locus specific probe, a 20q13 locus specific probe, a 21q22 locus specific probe, a 3q chromosome arm probe, a 5p chromosome arm probe, a 7p chromosome arm probe, a 3p chromosome arm probe, and a 20q chromosome arm probe.

An advantage of the invention is that it allows for the detection of lung cancer with improved sensitivity, as compared to conventional methods such as cytology. These probes and methods can thus allow for the early detection of lung cancer, e.g., at a pre-invasive stage.

Another advantage of the invention is that it allows for the detection of cancer cells based on genetic alterations, rather than gross morphological changes in cell structure. Genetic alterations can be detected at an early stage, e.g., before the occurrence of visually detectable changes in cell structure.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of a conflict in terminology, the present specification will control. In addition, the described materials and methods are illustrative only and are not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and the claims.

Brief Description of the Drawings

Figure 1 depicts a receiver operator characteristic (ROC) curve derived from FISH analysis of specimens from cancer positive and cancer negative patients. Sensitivity (y axis) and specificity (x axis; 1 – specificity) are depicted for cutoff values ranging from 1 to 10 cells per specimen.

Detailed Description of the Invention

The invention includes probe sets and methods of using probes and probe sets for the detection of lung cancer. The probes and methods described herein allow for the rapid and sensitive detection of lung cancer in a biological sample such as a bronchial specimen, a lung biopsy, or a sputum sample. In addition, the invention includes methods of selecting probe sets for the detection of cancer.

Chromosomal Probes

Suitable probes for *in situ* hybridization in accordance with the invention fall into three broad groups: chromosome enumeration probes, which hybridize to a chromosomal region and indicate the presence or absence of a chromosome; chromosome arm probes, which hybridize to a chromosomal region and indicate the presence or absence of an arm of a chromosome; and locus specific probes, which hybridize to a specific locus on a chromosome and detect the presence or absence of a specific locus. Chromosomal probes and combinations thereof are chosen for sensitivity and/or specificity when used in methods for the detection of lung cancer. Probe sets can include any number of probes, e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 probes.

A chromosome enumeration probe can hybridize to a repetitive sequence, located either near or removed from a centromere, or can hybridize to a unique sequence located at any position on a chromosome. For example, a chromosome enumeration probe can hybridize with repetitive DNA associated with the centromere of a chromosome. Centromeres of primate chromosomes contain a complex family of long tandem repeats of DNA, composed of a monomer repeat length of about 171 base pairs, that are referred to as alpha-satellite DNA. Non-limiting examples of chromosome enumeration probes

include probes to chromosomes 1, 3, 4, 6, 7, 8, 9, 10, 11, 12, 16, 17, and 18. Examples of several specific chromosome enumeration probes and their respective target regions are described in Table 1 of Example 1.

A chromosome arm probe can hybridize to a repetitive or unique sequence located on an arm, either the short or long arm, of a given chromosome. The gain or loss of the sequence to which the chromosome arm probe hybridizes can be used to indicate the gain or loss of the arm. Non-limiting examples of chromosome arm probes include probes to chromosome arms 3q, 5p, 7p, 3p, and 20q. Examples of specific chromosome arm probes and their respective target regions are described in Table 1.

A locus specific probe hybridizes to a specific, non-repetitive locus on a chromosome. Non-limiting examples of locus specific probes include probes to the following loci: 3p14; 3q26; 5p15; 5q31; 7p12; 8q24; 9p21; 10q23; 13q14; 17p13; 17q21; 20q13; and 21q22. Some of these loci comprise genes, e.g., oncgogenes and tumor suppressor genes, that are altered in some forms of cancer. Thus, probes that target these genes, either exons, introns, or regulatory sequences of the genes, can be used in the detection methods described herein. Examples of target genes include: FHIT (3p14); EGR1 (5q31); EGFR1 (7p12); c-MYC (8q24); PTEN (10q23); RB (13q14); P53 (17p13); and HER-2/neu (17q21).

Chromosomal probes can be of any size, but are typically about 50 to about $5x10^5$ nucleotides in length. Chromosomal probes can comprise repeated sequences, e.g., fragments of about 100 to about 500 nucleotides in length. Probes that hybridize with centromeric DNA and specific chromosomal loci are available commercially, for example, from Vysis, Inc. (Downers Grove, IL), Molecular Probes, Inc. (Eugene, OR), or from Cytocell (Oxfordshire, UK). Alternatively, probes can be made non-commercially from chromosomal or genomic DNA through standard techniques. For example, sources of DNA that can be used include genomic DNA, cloned DNA sequences such as a bacterial artificial chromosome (BAC), somatic cell hybrids that contain one, or a part of one, human chromosome along with the normal chromosome complement of the host, and chromosomes purified by flow cytometry or microdissection. The region of interest, e.g., a target region indicated in Table 1, can be isolated through cloning, or by site-specific amplification via the polymerase chain reaction (PCR). See, for example, Nath

and Johnson, Biotechnic Histochem., 1998, 73(1):6-22; Wheeless et al., Cytometry, 1994, 17:319-326; and U.S. Patent No. 5,491,224.

Chromosomal probes can contain a detection moiety that facilitates the detection of the probe when hybridized to a chromosome. Examples of detection moieties include both direct and indirect labels, as described below.

Chromosomal probes can be directly labeled with a detectable label. Examples of detectable labels include fluorophores, organic molecules that fluoresce after absorbing light of lower wavelength/higher energy, and radioactive isotopes, e.g., ^{32}P and ^{3}H. A fluorophore can allow a probe to be visualized without a secondary detection molecule. For example, after covalently attaching a fluorophore to a nucleotide, the nucleotide can be directly incorporated into the probe with standard techniques such as nick translation, random priming, and PCR labeling. Alternatively, deoxycytidine nucleotides within the probe can be transaminated with a linker. The fluorophore then is covalently attached to the transaminated deoxycytidine nucleotides. See, U.S. Patent No. 5,491,224.

Examples of fluorophores that can be used in the methods described herein are as follows: 7-amino-4-methylcoumarin-3-acetic acid (AMCA), Texas Red™ (Molecular Probes, Inc., Eugene, OR); 5-(and-6)-carboxy-X-rhodamine, lissamine rhodamine B, 5-(and-6)-carboxyfluorescein; fluorescein-5-isothiocyanate (FITC); 7-diethylaminocoumarin-3-carboxylic acid, tetramethylrhodamine-5-(and-6)-isothiocyanate; 5-(and-6)-carboxytetramethylrhodamine; 7-hydroxycoumarin-3-carboxylic acid; 6-[fluorescein 5-(and-6)-carboxamido]hexanoic acid; N-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a diaza-3-indacenepropionic acid; eosin-5-isothiocyanate; erythrosin-5-isothiocyanate; and Cascade™ blue acetylazide (Molecular Probes, Inc., Eugene, OR).

In methods using multiple probes, fluorophores of different colors can be chosen such that each chromosomal probe in the set can be distinctly visualized. Alternatively, two or more probes in a set can be labeled with the same or a similar fluorophore. Probes can be viewed with a fluorescence microscope and an appropriate filter for each fluorophore, or by using dual or triple band-pass filter sets to observe multiple fluorophores. See, for example, U.S. Patent No. 5,776,688. Alternatively, techniques

such as flow cytometry can be used to examine the hybridization pattern of the chromosomal probes.

Probes also can be indirectly labeled, e.g., with biotin or digoxygenin, although secondary detection molecules or further processing is required to visualize the labeled probes. For example, a probe labeled with biotin can be detected by avidin conjugated to a detectable marker, e.g., a fluorophore. Additionally, avidin can be conjugated to an enzymatic marker such as alkaline phosphatase or horseradish peroxidase. The enzymatic markers can be detected in standard colorimetric reactions using a substrate for the enzyme. Substrates for alkaline phosphatase include 5-bromo-4-chloro-3-indolylphosphate and nitro blue tetrazolium. Diaminobenzoate can be used as a substrate for horseradish peroxidase.

In Situ Hybridization

The presence or absence of cells with chromosomal aberrations is determined by *in situ* hybridization. Cells with chromosomal aberrations have, for example, an abnormal number of chromosomes and/or have chromosomal structural alterations such as the gain or loss (e.g., hemizygous or homozygous loss) of a specific chromosomal region, such as a locus or a chromosomal arm as indicated in Table 1. For example, a cell having one or more chromosomal gains, e.g., three or more copies of any given chromosome, can be considered to test positive in the methods described herein. Cells exhibiting monosomy and nullisomy may also be considered test positive under certain circumstances. In general, *in situ* hybridization includes the steps of fixing a biological sample, hybridizing a chromosomal probe to target DNA contained within the fixed biological sample, washing to remove non-specific binding, and detecting the hybridized probe.

A "biological sample" is a sample that contains cells or cellular material, e.g., cells or cellular material derived from pulmonary structures, including but not limited to lung parenchyme, bronchioles, bronchial, bronchi, and trachae. Non-limiting examples of biological samples useful for the detection of lung cancer include bronchial specimens, lung biopsies, and sputum samples. Examples of bronchial specimens include bronchial secretions, washings, lavage, aspirations, and brushings. Lung biopsies can be obtained

by methods including surgery, bronchoscopy, and transthoracic needle biopsy. In one example, touch preparations can be made from lung biopsies.

In addition, biological samples can include effusions, e.g., pleural effusions, pericardial effusions, or peritoneal effusions. In addition, biological samples can include cells or cellular material derived from tissues to which lung cancers commonly metastasize. These tissues include, for example, lymph nodes, blood, brain, bones, liver, and adrenal glands. Thus, the probes and probes sets described herein can be used to detect lung cancer and lung cancer metastasis.

Typically, cells are harvested from a biological sample and prepared using techniques well known to those of skill in the art. For example, cells can be harvested by centrifuging a biological sample, such as a bronchial washing, and resuspending the pelleted cells. Typically, the cells are resuspended in phosphate-buffered saline (PBS). After centrifuging the cell suspension to obtain a cell pellet, the cells can be fixed, for example, in acid alcohol solutions, acid acetone solutions, or aldehydes such as formaldehyde, paraformaldehyde, and glutaraldehyde. For example, a fixative containing methanol and glacial acetic acid in a 3:1 ratio, respectively, can be used as a fixative. A neutral buffered formalin solution also can be used, and includes approximately 1% to 10% of 37-40% formaldehyde in an aqueous solution of sodium phosphate. Slides containing the cells can be prepared by removing a majority of the fixative, leaving the concentrated cells suspended in only a portion of the solution. The cell suspension is applied to slides such that the cells do not overlap on the slide. Cell density can be measured by a light or phase contrast microscope.

Prior to *in situ* hybridization, chromosomal probes and chromosomal DNA contained within the cell each are denatured. If the chromosomal probes are prepared as a single-stranded nucleic acid, then denaturation of the probe is not be required. Denaturation typically is performed by incubating in the presence of high pH, heat (e.g., temperatures from about 70°C to about 95°C), organic solvents such as formamide and tetraalkylammonium halides, or combinations thereof. For example, chromosomal DNA can be denatured by a combination of temperatures above 70°C (e.g., about 73°C) and a denaturation buffer containing 70% formamide and 2X SSC (0.3M sodium chloride and 0.03 M sodium citrate). Denaturation conditions typically are established such that cell

morphology is preserved. For example, chromosomal probes can be denatured by heat, e.g., by heating the probes to about 73°C for about five minutes.

After removal of denaturing chemicals or conditions, probes are annealed to the chromosomal DNA under hybridizing conditions. "Hybridizing conditions" are conditions that facilitate annealing between a probe and target chromosomal DNA. Hybridization conditions vary, depending on the concentrations, base compositions, complexities, and lengths of the probes, as well as salt concentrations, temperatures, and length of incubation. For example, *in situ* hybridizations are typically performed in hybridization buffer containing 1-2X SSC, 50-55% formamide, a hybridization acceleratant (e.g. 10% dextran sulfate), and blocking DNA to suppress non-specific hybridization. In general, hybridization conditions, as described above, include temperatures of about 25°C to about 55°C, and incubation lengths of about 0.5 hours to about 96 hours. More particularly, hybridization can be performed at about 32°C to about 45°C for about 2 to about 16 hours.

Non-specific binding of chromosomal probes to DNA outside of the target region can be removed by a series of washes. Temperature and concentration of salt in each wash depend on the desired stringency. For example, for high stringency conditions, washes can be carried out at about 65°C to about 80°C, using 0.2X to about 2X SSC, and about 0.1% to about 1% of a non-ionic detergent such as Nonidet P-40 (NP40). Stringency can be lowered by decreasing the temperature of the washes or by increasing the concentration of salt in the washes.

Detection of Chromosomal Abnormalities

Gain or loss of chromosomes or chromosomal regions within a cell is assessed by examining the hybridization pattern of the chromosomal probe or set of chromosomal probes (e.g., the number of signals for each probe) in the cell, and recording the number of signals. In a typical assay, the hybridization pattern is assessed in a plurality of cells, e.g., about 25-5,000 cells.

Samples containing a plurality of cells, e.g., at least about 100, of which 1 or more, e.g., at least about 5, 6, 7, 8, 9, 10, 15, or 20, cells "test positive" typically are considered cancer positive. By "test positive" is meant possessing the gain or loss of a

chromosome, chromosomal arm, or locus as described herein. Criteria for "test positive" can include testing positive with one, two, three, four or more probes. In addition, "test positive" can include performing a hybridization analysis with multiple probes, e.g. four probes, and detecting abnormal hybridization patterns with a subset of the probes, e.g., at least two or three probes.

A sample containing cells, e.g. cells placed on a flat surface such as a slide, can be evaluated by a variety of methods and using a variety of criteria. The probes and methods described herein are not limited to usage with a particular screening methodology. For example, in what is known as the "scanning method," the observer scans hundreds to thousands of cells for cytologic abnormalities (as viewed with a DAPI filter). The number of cells assessed depends on the cellularity of the specimen, which varies from patient to patient. Cytologic abnormalities commonly but not invariably associated with neoplastic cells include nuclear enlargement, nuclear irregularity, and abnormal DAPI staining (frequently mottled and lighter). In the scanning method, the observer primarily focuses the evaluation of the cells for chromosomal abnormalities (as demonstrated by FISH) on those cells that also exhibit cytologic abnormalities. In addition, a proportion of the cells that do not have obvious cytologic abnormalities can be evaluated, since chromosomal abnormalities occur in the absence of cytologic abnormalities. The scanning method is described in further detail in U.S. Patent No. 6,174,681, the content of which is incorporated by reference.

Screening, Monitoring, and Diagnosis of Patients for Lung Cancer

The methods described herein can be used to screen individuals for lung cancer or to monitor patients diagnosed with lung cancer. For example, in a screening mode, individuals at risk for lung cancer, such as individuals who smoke or have been chronically exposed to smoke, or individuals chronically exposed to asbestos, are screened with the goal of earlier detection of lung cancer. In addition, the probes and methods described herein can be used for the diagnosis of symptomatic patients. The methods described herein can be used alone, or in conjunction with other tests. For example, a patient having an increased risk of lung cancer can be screened for lung cancer by performing *in situ* hybridization as described herein together with other

standard tests such as imaging analysis, e.g., CT, spiral CT, and X-ray analysis, and/or cytology. Alternatively, standard methods can be performed first on a patient, and if the standard test gives equivocal or negative results, then a method described herein can be performed.

The methods described herein can also be used to select a therapy for a patient diagnosed as having lung cancer. The methods can thus simultaneously diagnose a lung cancer and provide useful information as to possible treatments for the cancer. Several of the probes described herein are directed to oncogenes and tumor suppressor genes. If one or more of these genes is found to be altered in the course of a determination that the patient has cancer, then this information can be used to select a therapy, e.g., a therapy that modulates (increases or decreases) the presence or activity of these genes and/or their protein products. For example, if an alteration of the 17q21 locus is discovered, then this information could be used to design a Her-2-based therapy (see, e.g., Cragg et al., Curr. Opin. Immunol., 1999, 11:541-547). The loci containing specific oncogenes and tumor suppressor genes are indicated in Table 1.

Probe Selection Methods

The selection of individual probes and probe sets can be performed using the principles described in the examples. These selection methods make use of discriminate and/or combinatorial analysis to select probes and probes sets that are useful for the detection of lung cancer with high sensitivity.

The methods described herein preferably have a combined sensitivity and specificity that is better than that of conventional methods, particularly for the early detection of lung cancer. As described in the examples, 26 chromosomal probes were hybridized to 27 different lung tumor specimens and 12 normal adjacent tissue specimens, and the extent of gain and loss of each target was measured. To analyze this data and select the most useful probe sets, several rules were developed that, when considered in combination, yield probe sets having a high sensitivity and specificity. Each rule is not hard-and-fast but states general preferences that are weighed against the other rules in order to arrive at optimally performing probe sets.

(1) Each probe selected for a probe set should have an ability on its own to discriminate between tumor and normal tissue. Probes with high discrimination abilities are preferred. The discrimination analysis utilizes two different approaches: (a) comparing the means and standard deviations between the tumor specimen set and normal adjacent tumor specimen set of the percentage of cells with target gain and loss for each of the probe targets, and (b) calculating the sensitivity and specificity of each probe individually for identifying the tumor and normal adjacent tumor specimens, for various cutoff values of the cell percentages for targets gained and lost. Several different metrics can be generated to evaluate approach (a), which included calculation of D.V. (discriminate value), S.D.M. (standard deviation at "midpoint"), and p-value. D.V. and p-value are generally accepted methods for evaluation. The relevance of S.D.M. is that it is the cutoff value, as a multiple of the standard deviations from the tumor and normal means, at which the sensitivity would equal the specificity if the means and standard deviations actually equaled the true values of the two populations. For example, if the midpoint was one standard deviation of the tumor specimens from the mean of the tumor specimens, and one standard deviation of the normal adjacent specimens from the mean of the normal adjacent specimens, then the sensitivity and specificity would each equal 84% (this also assumes normal-error distributions for each population, which is less likely to be true for the normal adjacent tissue distributions due to their proximity to 0). The larger the S.D.M. the greater the sensitivity and specificity of that probe.

(2) The primary metric for combined sensitivity and specificity will be the quantity called 'vector' which is the magnitude of the vector drawn between the points on a sensitivity versus specificity plot representing the ideal (sensitivity = specificity = 1) and the measured sensitivity and specificity. Therefore the vector value ranges from 0 for the ideal case and 1.414 for the worst case.

(3) Each probe selected for a probe set should complement the other selected probes, that is, it should identify additional tumor specimens that the other probe(s) failed to identify. One method of identifying the best complementing set of probes is to take the probe with the lowest vector value, remove the group of tumor specimens it identified from the full set of tumor specimens, and then determine the probe with lowest vector value on the remaining tumor specimens. This process can be continued as necessary to

complete the probe set. The approach selected here of generating all possible probe combinations, and calculating the sensitivity and specificity of each, predicts the performance of all possible probe sets and allows selection of the minimal probe set with the highest performance characteristics. Also, a variety of combinations with similarly high performance characteristics is obtained. Considering the possible errors due to the finite number of specimens tested, several of the high ranking probe combinations can be compared based on other practical characteristics such as relevance to disease prognosis or difficulty in making the probe.

(4) The ability of probes to complement one another is more important than the discriminating ability of individual probes, except as indicated in (5) below.

(5) Regardless of the measured ability to complement other probes, each probe must identify a statistically different percentage of test positive cells between the tumor and normal adjacent tissue specimen sets. If this condition is not met then a probe might be selected erroneously based on apparent complementation.

(6) Data for combinations of two probes is more reliable than data for combinations of three probes, and data for combinations of three probes is more reliable than data for combinations of four probes. This results from the reduced ability to make correlations between greater numbers of probes with the finite number of specimens tested.

(7) The dependence of probe and probe combination performance as a function of cutoff value must be considered. "Cutoff value" refers to the percentage of cells in a population that must have gains or losses for the sample to be considered positive. A sample is therefore called as positive or negative depending upon whether the percentage of cells in the sample is above the cutoff value or equal to or less than the cutoff value.

In general, the combined specificity and sensitivity of probes is better at low cutoff values. However, when the cancer cells are distributed within a matrix containing many normal cells, such as bronchial secretions or sputum, probes performing best at high cutoffs are more likely to be detected. This is because good performance at high cutoffs indicates a higher prevalence of cells containing the abnormality. Examples of cutoff values that can be used in the calculations include about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, and 60%.

(8) The measurement of target gain is favored over measurement of target loss. Overlapping targets or poor hybridization to some cells can falsely suggest monosomy. Locus-specific or chromosomal arm probes designed to detect deletions are generally smaller than locus-specific or chromosomal arm probes designed to detect gain since the deletion probes must not extend beyond the minimally deleted region. If too much of the "deletion probe" extends beyond the deleted sequence, enough signal may remain to be falsely counted. Since "deletion probes" are usually kept small the signals are not as intense as signals for targets typically gained. This in turn makes it more likely that real signals from targets being monitored for deletion may be miscounted. Likewise, repetitive sequence probes, like some chromosome enumeration probes used here are preferable to single locus probes because they usually provide brighter signals and hybridize faster than locus specific probe. On the other hand, repetitive sequence probes are more sensitive to polymorphisms than locus specific probes.

(9) A probe or combination of probes preferably shows an improvement over conventional methods such as cytology. A probe or probe combination preferably detects lung cancer with a sensitivity of at least about 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or even 100%. A probe or probe combination preferably detects lung cancer with a vector value of less than about 0.500, 0.450, 0.400, 0.350, 0.300, 0.250, 0.200, 0.150, or 0.100.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1: Probe Selection

A collection of 26 probes was assembled as candidates for detecting chromosomal abnormalities in lung cancer by *in situ* hybridization. The probes were hybridized to a collection of lung tumor touch preparations, and the distribution of the copy number per cell of each probe target was determined. In order to conserve tumor specimens, multi-color hybridizations were utilized to limit the number of hybridization regions per specimen to 8. To achieve this, the 26 probes were labeled with several different

fluorophores. Mixtures of 3 or 4 probes each were prepared from the labeled probes forming the 8 probe sets. Where possible, chromosome enumeration probes and locus specific probes that target the same chromosome were combined in the same set to distinguish whole chromosome aneuploidy from gains and losses of regions within a chromosome.

The 26 probes selected for hybridization to lung touch preparations are described in Table 1. The probes included 13 chromosome enumeration probes (CEP™ probes from Vysis, Inc.; targeting repetitive centromeric sequences) and 13 locus specific probes (LSI™ from Vysis, Inc. or BAC preparations; targeting unique sequences associated with amplified or deleted chromosomal regions). Column 3 of Table 1 describes the target location of each of the 26 probes. For several of the probes, oncogenes or tumor suppressor genes that are located at the relevant locus are also listed.

Mixtures of 3 probes, labeled with SpectrumAqua™, SpectrumGreen, and SpectrumOrange™, or 4 probes, labeled with SpectrumAqua™, SpectrumGreen™, SpectrumGold™, and SpectrumRed™, were prepared to form the 8 probe sets. The fluorescent label used for each probe and the probe set containing each probe are described in columns 4 and 5, respectively, of Table 1.

Tumor touch preparations, prepared from lung tumors removed from 27 patients with a range of lung cancers, were used for testing the 26 probes. In addition, specimens prepared from normal lung tissue generally at some distance from the tumors (NAL = normal adjacent lung tissue) from twelve of the same patients were also tested in order to examine the background levels of gained and lost targets for each probe. The characteristics of the lung tumor and normal specimens are listed in Table 2. Touch preparations were prepared by pressing a piece of lung tumor or normal adjacent tissue against a glass microscope slide and fixing briefly in ethanol. The specimens were then stored at -20°C until ready for use.

Prior to *in situ* hybridization, the touch preparations were treated to improve *in situ* hybridization performance by the following protocol.

(1) Fix the specimen slide in a fresh Carnoy's solution (3:1 methanol:acetic acid) for 20 minutes at room temperature. Allow the slide to dry in the air.

(2) Place the slide on a 45°C hot plate for 15 minutes.

(3) Incubate the slide in 2xSSC at 37°C for 10 minutes.

(4) Place the slide in a pepsin solution (0.05 mg pepsin per ml 10 mM HCl) at 37°C for 13 minutes. The pepsin solution is prepared fresh each day by diluting 25 µL of a pepsin stock solution (100 mg pepsin/mL water; use 2,500-3,000 U/mg pepsin) into 50 mL of 10 mM HCl.

(5) Place the slide in 1xPBS for 5 minutes at room temperature.

(6) Fix the slide in 1% formaldehyde for 5 minutes at room temperature. The formaldehyde solution is prepared by mixing 1.35 mL of 37% formaldehyde with 48.15 mL of 1xPBS and 0.5 mL of 2 M $MgCl_2$. Discard after each day of use.

(7) Place the slide in 1xPBS for 5 minutes at room temperature.

(8) Dehydrate the specimen by placing the slide in a series of ethanol solutions (70%, 85%, 100%), 1-5 minutes per solution. Allow the specimen to dry in the air before denaturing.

After performing the above treatments, fluorescence *in situ* hybridization was performed on all specimens as follows.

(1) Denature the specimen's DNA by placing the slide in a solution of 70% formamide/2xSSC at 73°C for 5 minutes.

(2) Dehydrate the specimen by placing the slide in a series of ethanol solutions (70%, 85%, 100%), 1-5 minutes per solution. Allow the specimen to air dry before applying denatured probe.

(3) Denature a probe solution by placing a tube containing the probe in a 73°C water bath for 5 minutes.

(4) Apply the denatured probe solution to the denatured slide, place a coverslip over the solution, and seal the coverslip by applying rubber cement along the edges. Allow the probe to hybridize overnight at 37°C in humidified chamber.

(5) Wash the slide in a Coplin jar in 0.4xSSC/0.3% NP-40 for 3 minutes at 70°C (or 1 minute at 73°C). Wash 4 slides simultaneously per Coplin jar.

(6) Soak the slide in 2xSSC/0.1% NP-40, for several seconds to several minutes.

(7) Apply antifade/counterstain solution and cover with a coverslip. Store the slides at -20°C until analyzed.

Hybridized specimen slides were viewed on a fluorescence microscope using single bandpass filter sets specific for each of the 4 fluorescent labels and the DAPI counterstain. Each touch preparation was analyzed by counting the number of spots of each fluorescent color in 100 consecutive non-inflammatory cells and the copy number of each probe target recorded. Several of the specimens did not hybridize well with all 26 probes, so the number of specimens tested differs for each probe. In addition, probe set 8 was not tested on all specimens.

Example 2: Analysis of *In Situ* Hybridization Data

The target copy number data for each of the normal and tumor specimens was analyzed for the ability of each probe to discriminate between tumor and normal specimens (discriminate analysis) and for the ability of probe combinations to discriminate between tumor and normal specimens (combinatorial analysis). These analyses were used as part of the data considered in deciding which probes should be used individually or in concert to best identify lung cancer cells.

Discriminate Analysis

The ability of individual probes to discriminate between the normal specimen group and the tumor specimen group was evaluated first by comparing the averages and standard deviations of the percentages of abnormal cells found in each group. These data are listed in Tables 3 (normal specimen group) and 4 (tumor specimen group). The first 26 rows in each table lists data derived from absolute target counts per cell, for each of the 26 probes tested. For these calculations, individual targets present in greater than 2 copies were considered an abnormal gain in copy number, and targets present in less than 2 copies were considered an abnormal loss in copy number. The last 8 rows in Tables 3 and 4 list data derived from ratios of LSI/CEP target numbers, or in the case of chromosome 5, the ratio of LSI 5p15/LSI 5q31 target numbers. Ratios were only calculated when both probes were contained in the same probe set. The ratios were calculated on a cell-by-cell basis. For the purpose of these calculations, cells were considered to have target gain when ratios were greater than 1, and target loss when ratios were less than 1.

In Tables 3 and 4, the columns headed 'Ave. % cells ...' are the averages of the percentage of cells found in each specimen with either target copy number gain or target copy number loss, as indicated in the heading. The columns headed 'S.D. % cells ...' are the standard deviations of the average cell percentages for the number of specimens ('Number of specimens ....' columns) in which interpretable hybridizations for each specific probe were obtained.

Included in Table 4 are three columns containing different measures of the ability of each probe to discriminate between the tumor and normal specimen groups. The discriminate value, D.V., is calculated according to Equation 1:

$$DV = (M_T - M_N)^2/(SD_T^2 + SD_N^2) \quad (1)$$

with values being larger for greater separation between the mean of the normal specimens, M_N, and the mean of the tumor specimens, M_T, and for smaller standard deviations of the normal, $S.D._N$, and tumor, $S.D._T$, specimens.

The 'SD's at midpoint', S.D.M. is calculated by Equation 2:

$$S.D.M. = (M_T - M_N)/(SD_T + SD_N) \quad (2)$$

and is the number of standard deviations from the tumor and normal group means which equal the separation of the means. If the means and standard deviations were the true values for the tumor and normal populations, then S.D.M. is the point at which the sensitivity and specificity are equal to each other. The larger the S.D.M., the greater the value of the sensitivity and specificity.

The third measure of discrimination listed in Table 4 is the probability, p, that the measured means are from the same population. The value of p is determined from the Student's t-test. In effect the smaller the p value, the more statistically different the tumor population is from the normal population. A $p < 0.05$ is typically considered to represent a statistically significant difference between the two groups.

The p values in Table 4 indicate that all of the 26 probes found statistically significant ($p < 0.05$) gains for the tumor specimen group relative to the normal group,

when using the absolute target numbers. When viewed as ratios between LSI and corresponding CEP or LSI target numbers, 5 of the 8 ratios showed significant differences (last 8 rows in Table 4). By contrast, only 2 of the 26 probes found statistically significant loss of absolute target numbers (LSI 8p24 and CEP 17), while 5 of the 8 ratios showed significant differences.

The rows of Table 4 are sorted from highest to lowest D.V. for gain of targets. The data derived from absolute target counts is sorted separately from the ratio data. Examination of the D.V., S.D.M., and p values for target gain shows relatively good correspondence between the three discrimination parameters. The top 5 discriminating probes selected by all three parameters are the same, LSI 5p15, LSI 7p12, CEP 1, CEP 6, and LSI 8q24, in descending order (all indicating gain of targets in tumor specimens).

Another approach within the overall selection method for determining which probes provide the best discrimination between normal and tumor specimens is to look at the number of specimens correctly identified by each probe. This requires selecting a cutoff number for the percentage of cells with gains or losses. A sample is then called positive or negative for cancer depending upon whether the percentage of cells in the sample is above the cutoff value or equal to or less than the cutoff value, respectively. The accuracies of identifying the positive samples (sensitivity) and negative samples (specificity) are then used to select the best probes.

Table 5 lists the specificity and sensitivity of gain and loss of all 26 probe targets and the same CEP/LSI and 5p/q ratios listed in Tables 3 and 4. The table includes the specificity and sensitivity values at 6 different cutoff values (5%, 10%, 20%, 30%, 40%, and 50%). The table also includes two measures of the combined specificity and sensitivity, since the overall ability to discriminate between tumor and normal specimens depends on both specificity and sensitivity. The first combined attribute is the product of specificity and sensitivity. The product is largest if both specificity and sensitivity are high, and is reduced if either or both are low. The other combined attribute, designated as "vector," is calculated according to Equation 3:

$$\text{Vector} = [(1\text{-specificity})^2 + (1\text{-sensitivity})^2]^{0.5} \qquad (3)$$

This attribute has a value of 0 when specificity and sensitivity = 1, and increases to 1.414 as both approach 0.

The rows in Table 5 are sorted by increasing vector value for each cutoff value. The data derived from absolute target counts is sorted separately from the ratio data. Target gains dominate the top of the table and the same probes tend to show the lowest vector values, although their relative order changes with cutoff value. Probes showing consistently high discrimination ability based on the vector value and absolute target counts include LSI 8q24, LSI 5p15, LSI 7p12, LSI 3q26, LSI 20q13, LSI 5q31, LSI 3p14, LSI 17q21, CEP 1, CEP 4, CEP 6, CEP 7, CEP 9, and CEP 16. Each of these probes is found in the top 10 rows for at least two of the cutoff values. The target ratios generally showed lower vector values except for the chromosome 5p15/5q31 ratio which had vector values comparable to some of the best probes based on their absolute target counts.

Combinatorial Analysis

The ability of multiple probes used in concert to increase assay sensitivity (complementation) was investigated using combinatorial analysis. The analysis was initiated by generating all possible combinations of a group of probes. The counting data from each specimen was then examined to determine if any of the probes in each combination identified gain or loss of their target above a threshold number of cells. If any of the probes in a combination were positive, then the specimen was considered positive for cancer for that combination.

The combinations were kept to a maximum of four probes. The entire set of 26 probes was not used to generate all combinations due to the large number of possible combinations that would be generated for the 26 probes and their relevant ratios, each of which would be examined for gain and loss (866,847 possible combinations of 1, 2, 3, and 4 probes). Instead, the set of probes and ratios was reduced to include only those probes that identified gains, and those probes that identified losses with $p < 0.01$ (Table 4). This provided some assurance that probe combinations would not be over rated as a result of randomly high target counts of individual probes. To further reduce complexity, two different groups of probes were examined separately. Group 1 included

all of the probes for which the absolute counts identified target gain or loss with $p < 0.01$. Group 2 replaced the members of Group 1 with their corresponding LSI/CEP or LSI/LSI ratio, if the ratio identified target gain or loss with $p < 0.01$. Therefore, Group 1 consisted of all of the probes for gain listed in the first 25 rows of Table 4 (because of its high significance, LSI 5p15/LSI 5q31 was also included in this group) and none of the probes for losses. Group 2: replaced LSI 7p12 and LSI 8q24 with LSI 7p12/CEP 7 and LSI 8q24/CEP 8, respectively, for gains; deleted the other LSI probes that had corresponding LSI/CEP ratios with $p > 0.01$; and added LSI 9p21/CEP 9 and LSI 17p13/CEP 17 for loss.

Tables 6 through 9 list the combinations of 2, 3, and 4 probes with the combined highest sensitivities and specificities, for cutoff values of 10% (Table 6), 20% (Table 7), 30% (Table 8), and 40% (Table 9), respectively. The measure of combined sensitivity and specificity used to order the combinations was the vector value. A particular combination was excluded from the tables if a subset of probes in the combination gave an equal or lower vector value. The probes contributing to the best combinations changed as the cutoff value was increased. The best vector values also increased as the cutoff was increased, as seen previously in Table 5 for single probes. In determining the number of probes in a combination, ratios were counted as two probes, unless one of the probes in the ratio was also in the combination. In general, ratios were not found in the better scoring combinations, except for the LSI 5p15/LSI 5q31 ratio. Also, target loss rarely ranked in the top performing probe combinations. As a result, in further discussion the gain of a target is implied, unless specifically denoted as a loss.

At a percent cell cutoff value of 10% (Table 6), LSI 8q24 and LSI 5p15 were commonly found in the top performing combinations of two probes, and complemented each other as well. LSI 8q24 was also complemented well by LSI 17q21, LSI 5q31, LSI 9p21, CEP 1, CEP 6, CEP 7, CEP 9, CEP 11, and CEP 17. LSI 5p15 was also complemented well by LSI 17q21, LSI 5q31, LSI 9p21, LSI 13q14, CEP 8, CEP 12, and CEP 17.

In addition, LSI 7p12 and CEP 1 complemented one another well. The same probes were also found in the better combinations of three and four probes.

When the cutoff was increased to 20% (Table 7), LSI 5p15 remained in the top combinations of two, and was complemented best by LSI 3q26, CEP 16, LSI 20q13, LSI 17q21 and CEP 4. LSI 8q24, LSI 3p14, LSI 5q31, LSI 7p12, CEP 3, CEP 6, and CEP 9 also provided good complementation to LSI 5p15. LSI 8q24 fell lower in the list, although still a good performer, being complemented by LSI 7p12 and CEP 6. The better combinations of three and four probes also included these probes as well as other probes identified above in the better combinations at the cutoff of 10.

As the cutoff was increased to 30% (Table 8), LSI 5p15 persisted in the better combinations, and LSI 8q24 was absent from the higher-ranking combinations. Complementation of LSI 5p15 was provided by CEP 6, CEP 16, LSI 20q13, LSI 3q26, LSI 17q21, LSI 7p12, and LSI 3p14. Also, LSI 7p12 was complemented by CEP 6, and CEP 6 and CEP 7 complemented one another. Detection of target loss was only found to be important in combinations of four probes (LSI 17p13 loss relative to CEP 7).

Increasing the cutoff value to 40% (Table 9) reduced the importance of LSI 5p15 in two probe combinations, and placed LSI 7p12 at the top of the list, which was complemented best by LSI 3q26 and CEP 6, and also by CEP 18, CEP 4, CEP 16, LSI 20q13 and LSI 5p15. CEP 6 ranked high when complemented by either CEP 1 or CEP 7. Other high ranking pairs of probes included LSI 3q26 with either LSI 5p15, CEP1, or CEP 7. In combinations of three probes, the combination of LSI 7p12 and CEP 6 with CEP 11 was at the top of the list, just ahead of combinations of CEP 6 with either CEP 1 or CEP 7, also complemented by CEP 11. Other probes included in the better performing combinations of three were 17q21, LSI 3q26 CEP 4, CEP 16, CEP 18, and LSI 20q. In combinations of four probes, CEP 6 combined with either CEP 1 or CEP 7 was at the top of the list when complemented by 17p13/CEP 16 loss. Another loss, 9p21/CEP 9 was next when combined with CEP 7 and LSI 3q. Other high ranking combinations of four included LSI 7p12, LSI 10q23, CEP 10, CEP 11, LSI 5p15, LSI 5q31, LSI 5p/LSI 5q, CEP 6, CEP 7, and CEP 9.

Example 3: Selection of Probe Sets

Table 13 lists probes and probe sets selected by analyzing the data from the discriminate and combinatorial analyses and applying the probe selection criteria

described herein. The probe sets of Table 13 range in size from a single probe to 4 probes. Assays using additional probes, e.g., more than four, and additional fluorescent labels can be performed.

The single probes listed in Table 13 are the probes that individually showed improvement over cytology. These include LSI 5p15, LSI 7p12, LSI 8q24, CEP 1, CEP 6, and CEP 9. For each of these probes, the vector value was less than 0.400 for two of the cutoff values tested. Other probes described herein also gave vector values less than 0.400 for a single cutoff. However, good performance for two cutoff values implies that a probe is more robust.

Next, Table 13 lists 2-probe combinations. The probe pairs placed in this group were required to have a vector value less than 0.400 and rank in the top approximately 30 probe pairs (lowest vector values) for at least one cutoff value. The vector values are listed in the table for each probe pair for each cutoff value in which the probe pair was ranked in the top 30. Of special note are the probe pairs of LSI 5p15 + LSI 8q24, LSI 5p15 + CEP 12, and LSI 5p15 + LSI 17q21 which have vector values less than 0.400 at 3 different cutoff values.

Next, Table 13 lists 3-probe combinations. Only a few combinations of 3 probes are listed under this heading since these are the few sets that improved over combinations of 2 probes for any particular cutoff value.

Next, Table 13 lists 4-probe combinations. Only one combination of 4 probes is listed under this heading since it was the only combination that improved over the combinations of 2 and 3 probes for any particular cutoff value.

To take advantage of the practical capability of using 3 and 4 FISH probes together, a strategy of redundancy can be introduced. Under this strategy, a third probe could be added to a pair of complementary probes if it also complemented one of the 2 probes. Alternatively, it might not complement either probe well, but instead it might be the next highest performing single probe. Similarly, 4 probe pairs could be generated by combining pairs of complementary probes. Some 3 and 4 probe sets generated using redundancy of the 2-probe sets listed in Table 13 are listed in a lower part of the same table. An alternative approach is to pick a 2-probe pair and add an additional 2 probes, one of which complements one member of the first pair, and the other of which

complements the other member of the first pair. One benefit of redundancy probes is that assay specificity might be improved by requiring 2 of the targets to be gained in order to call the specimen abnormal. Redundancy can also improve sensitivity since if one probe hybridization should fail in an assay, the redundant probe might still detect the target gain. Other practical issues can be considered in probe selection. For example, the 4 probe set of LSI 5p15 + LSI 8q24 + LSI 7p12 + LSI 17q21 can be constructed from probes in three of the top performing combinations of 2 probes listed in Table 13. The significance of this probe set is that it detects two loci of therapeutic importance, 17q21 containing the HER-2/neu gene and 7p12 containing the epidermal growth factor receptor gene (EGFR). The identification of abnormalities at these loci can be used to select an appropriate treatment regimen.

Example 4: Lung Cancer Detection

Two 3-color probe sets were chosen for preliminary testing on a series of bronchial secretion specimens. The results of this study showed that specificity and sensitivity equivalent to or better than conventional cytology could be obtained with multi-color FISH panels.

The results of the hybridizations of 3-color probe sets to each of 21 bronchial secretion smears are listed in Table 10, together with specimen identification numbers, clinical diagnoses, cytology results, and bronchoscopic biopsy results (two results when additional biopsy was performed). Each specimen was hybridized with two different 3-color probes sets. The first 3 color probe set contained LSI 8q24, LSI 5p15, and CEP 1, and the second set contained LSI 8q24, LSI 5p15, and CEP 6. Gain of the 5p15 target was found in 13 of the 13 FISH positive specimens. Gain of the 8q24, CEP1, and CEP 6 targets were found in 11, 7, and 5 of the 13 FISH positive targets, respectively. One of the specimen slides could not be evaluated by FISH due to poor morphology and no FISH abnormalities were found in the remaining 7 specimens. The performance of conventional cytology and FISH are compared to the clinical diagnosis in Tables 11 and 12, respectively. Clinical diagnosis was based on the combined information available to the clinician, and did not include the FISH result.

In the above methods, smears of bronchial secretions were prepared by placing a specimen between two microscope slides and sliding the slides apart from one another while applying slight pressure. The slides were then fixed briefly with ethanol and stored at -20° C until ready for use.

Smears of bronchial secretions were prepared for *in situ* hybridization by the following protocol.

(1) Incubate the specimen slide in 2xSSC at 37° C for 10 minutes.

(2) Place the slide in a pepsin solution (0.05 mg pepsin per mL 10 mM HCl) at 37°C for 13 minutes.

(3) Place the slide in 1xPBS for 5 minutes at room temperature.

(4) Fix the specimen by placing the slides in 1% formaldehyde for 5 minutes at room temperature.

(5) Place the slides in 1xPBS for 5 minutes at room temperature.

In Situ Hybridization was performed on the specimens as follows.

(1) Denature the specimen DNA by placing the slides in a solution of 70% formamide/2xSSC at 73° C for 5 minutes.

(2) Dehydrate the specimen by placing the slide in a series of ethanol solutions (70%, 85%, 100%), 1-5 minutes per solution. Allow the specimen to air dry before applying denatured probe.

(3) Denature a probe solution by placing a tube containing the probe in a 73°C water bath for 5 minutes.

(4) Apply the denatured probe solution to the denatured slide, place a coverslip over the solution, and seal the coverslip by applying rubber cement along the edges.

(5) Allow the probe to hybridize overnight at 37°C in humidified chamber.

(6) Wash the slide in a Coplin jar in 0.4xSSC/0.3% NP-40 for 3 minutes at 70°C (or 1 minute at 73°C). Wash 4 slides simultaneously per Coplin jar.

(7) Soak the slide in 2xSSC/0.1% NP-40 for several seconds to several minutes.

(8) Apply antifade/counterstain solution and cover with a coverslip. Store the slide at -20°C until analyzed.

Bronchial secretion smears were analyzed by scanning the entire specimen. Each microscope field was viewed sequentially with the 4 single bandpass filter sets (DAPI

Table 1 Probes Used for Probe Selection

PROBE NAME	DNA SOURCE	TARGET LOCATION	LABEL	PROBE SET
CEP 1, sat. II/III	Vysis product	1q12	SpectrumGreen	5
CEP 3, alpha sat	Vysis product	D3Z1, 3p11.1-q11.1	SpectrumAqua	6
LSI 3p14/FHIT	BAC	3p14	SpectrumOrange	6
LSI 3q26/TERC	BAC	3q26	SpectrumGreen	8
CEP 4, alpha sat.	Vysis product	4p11-q11	SpectrumAqua	8
LSI D5S721, D5S23	Vysis product	D5S721, D5S23, 5p15	SpectrumGreen	4
LSI EGR1	Vysis product	5q31	SpectrumOrange	4
CEP 6, alpha sat.	Vysis product	D6Z1, 6p11.1-q11	SpectrumGreen	6
CEP 7, alpha sat.	Vysis product	D7Z1, 7p11.1-q11.1	SpectrumAqua	5
LSI EGFR	BAC	7p12	SpectrumOrange	5
CEP 8, alpha sat.	Vysis product	D8Z2, 8p11.1-q11.1	SpectrumAqua	2
LSI c-myc	Vysis product	8q24	SpectrumOrange	2
CEP 9, alpha sat.	Vysis product	9p11-q11	SpectrumGreen	3
LSI 9p21	Vysis product	9p21	SpectrumGold	3
CEP 10, alpha sat	Vysis product	10p11.1-q11.1	SpectrumGreen	7
LSI 10q23 (PTEN)	BAC	10q23	SpectrumOrange	7
CEP 11, alpha sat.	Vysis product	D11Z1, 11p11.1-q11	SpectrumAqua	3
CEP 12, alpha sat.	Vysis product	D12Z3, 12p11.1-q11	SpectrumAqua	4
LSI 13/RB1 retinoblastoma 1	Vysis product	13q14	SpectrumGreen	2
CEP 16, sat. II	Vysis product	D16Z3, 16q11.2	SpectrumGold	8
CEP 17, alpha sat.	Vysis product	D17Z1, 17p11.1-q11.1	SpectrumAqua	1
LSI p53	Vysis product	17p13	SpectrumOrange	1
LSI her2/neu (ERBB2)	Vysis product	17q21	SpectrumGreen	1
CEP 18, alpha sat.	Vysis product	D18Z1, 18p11.1-q11.1	SpectrumAqua	7
LSI 20q13 (ZNF217)	Vysis product	20q13	SpectrumRed	8
LSI 21	Vysis product	D21S259, D21S341, D21S342, 21q22	SpectrumRed	3

Table 2 Lung Tumor and Normal Adjacent Tissue used for Probe Selection

SPECIMEN NAM	SPECIMEN TYPE	TUMOR TYPE	TUMOR GRADE
T1	tumor	bronchial alviolar carcinoma	2
T2	tumor	adenocarcinoma	2
T3	tumor	adenocarcinoma	2
T7	tumor	adenocarcinoma	4
T8	tumor	bronchial alviolar carcinoma	1
T9	tumor	adenocarcinoma	2
T10	tumor	adenocarcinoma	3
T11	tumor	squamous cell carcinoma	4
T12	tumor	adenocarcinoma	3
T13	tumor	large cell carcinoma	4
T14	tumor	adenocarcinoma	4
T15	tumor	carcinoid tumor	?
T16	tumor	adenocarcinoma	3
T17	tumor	adenocarcinoma	2
T18	tumor	large cell carcinoma	4
T19	tumor	adenocarcinoma	4
T20	tumor	squamous cell carcinoma	4
T21	tumor	squamous cell carcinoma	4
T22	tumor	squamous cell carcinoma	4
T23	tumor	adenocarcinoma	3
T24	tumor	adenocarcinoma	3
T25	tumor	squamous cell carcinoma	4
T26	tumor	adenocarcinoma	3
T27	tumor	adenocarcinoma	2
T28	tumor	?	?
T31	tumor	?	?
T32	tumor	?	?
N1	NAT	NA	NA
N2	NAT	NA	NA
N3	NAT	NA	NA
N7	NAT	NA	NA
N8	NAT	NA	NA
N12	NAT	NA	NA
N13	NAT	NA	NA
N14	NAT	NA	NA
N15	NAT	NA	NA
N16	NAT	NA	NA
N17	NAT	NA	NA
N18	NAT	NA	NA

*NAT = normal tissue adjacent to tumor tissue, NA = not applicable, ? = status unknown

Table 3 DISCRIMINATION ANALYSIS

PROBE	Number of specimens	Ave. % cells with gain	S.D. % cells with gain	Ave. % cells with loss	S D. % cells with loss
LSI 5p15	10	4.4000	2.8752	2.8000	1.9322
LSI 7p12	10	5.5500	2.4771	1.3000	1.9465
CEP 1	10	3.5500	0.8317	3.3000	2.5408
CEP 6	10	1.9000	2.2336	4 8000	2 5734
LSI 8q24	10	2.7500	1.9329	3.1000	1 8529
LSI 20q	10	3.9000	2.2336	4.5000	2.8771
CEP 9	10	2.1000	2.0790	7.1000	5.0211
LSI 3p14	10	4.3000	4 2439	2.9000	2.2828
CEP 16	10	2.8000	1.4757	10.1000	4 6774
CEP 4	10	2 8000	2 6162	2.6000	1 5055
LSI 3q	10	7.5000	3.2404	2 9000	3 0350
CEP 7	10	1.4000	0.9661	2.4000	2 0111
LSI 17q21	10	2.9000	2.4698	6.5000	2.8771
LSI 5q31	10	3.4000	1.6465	4 4000	2.5033
CEP 3	10	1.7000	1.4181	3 7000	2 2632
CEP 10	10	1.4000	2.0656	4.1000	2 9981
CEP 11	10	2.6500	2.3576	4.4000	1.7764
CEP 8	10	1.0000	1.0541	4.5000	2.9907
CEP 18	10	1.8000	1.9889	7.9000	3.8427
LSI 13	10	2.4500	2.2417	3.6500	2.7894
LSI 9p21	10	2.7500	2.8211	4.0000	3.0185
LSI 10q23	10	6.0000	4.5947	3.0000	2.1602
CEP 12	10	1.5000	1.2693	4 4000	2.6331
CEP 17	10	2.3000	2.6687	10 9000	4.4585
LSI 17p13	10	4 1000	3.9567	6 9000	3.2472
LSI 21	10	7.8500	5.8407	6 1500	4 9668
Ratios:					
5 p/q imbal.	10	5.2234	3.4875	3.2132	1.7602
LSI 7p12/CEP 7	10	6.3000	3.6833	1.1500	2.1350
LSI 8q24/CEP 8	10	6.1561	3.3667	2.9540	1 7098
LSI 3p14/CEP 3	10	7.0000	4.9666	3 6000	2 6331
LSI 17q21/CEP	10	11.4000	5.4610	6.2000	2.1499
LSI 10q23/CEP	10	8.5000	5.9489	3 0000	1.6997
LSI 9p21/CEP 9	10	7.7041	7.4657	3.9041	3 9546
LSI 17p13/CEP	10	11.3000	5.3759	6 0000	3 0551

Table 4 DISCRIMINATION ANALYSIS

PROBE	Number of specimens	Ave % cells with gain	S D % cells with gain	D V gain	S D M gain	p gain	Ave % cells with loss	S D % cells with loss	D V loss	S D M point - loss	p loss
LSI 5p15	26	34 0385	25 1483	1 3710	1 0576	0 000003	1 4615	1 9022	0 2437	-0 3491	0 079773
LSI 7p12	26	30 1154	21 6505	1 2708	1 0181	0 000005	1 3462	2 2617	0 0002	0 0110	0 952130
CEP 1	26	27 7308	21 7946	1 2292	1 0687	0 000007	7 7308	19 0527	0 0531	0 2052	0 256410
CEP 6	26	27 8462	24 2976	1 1307	0 9779	0 000012	3 7692	3 2901	0 0609	-0 1758	0 332295
LSI 8q24	27	22 7407	19 3621	1 0555	0 9388	0 000013	1 5926	1 5753	0 3842	-0 4397	0 038297
LSI 20q	19	26 2632	22 4297	0 9843	0 9067	0 000395	2 6842	2 1616	0 2546	-0 3604	0 100820
CEP 9	26	19 6923	17 6176	0 9834	0 8932	0 000031	6 6154	8 2998	0 0025	-0 0364	0 832828
LSI 3p14	26	21 5385	17 1912	0 9477	0 8042	0 000043	4 2692	6 6547	0 0379	0 1532	0 365082
CEP 16	19	21 9474	20 5520	0 8635	0 8692	0 000741	8 3684	5 9368	0 0525	-0 1631	0 398174
CEP 4	19	20 7368	19 5756	0 8248	0 8083	0 000888	2 9474	1 9571	0 0198	0 1003	0 600639
LSI 3q	19	29 7895	24 3278	0 8248	0 8085	0 000889	2 6316	5 8709	0 0016	-0 0301	0 872277
CEP 7	26	23 1154	24 0704	0 8126	0 8673	0 000106	2 6154	2 8576	0 0038	0 0442	0 801649
LSI 17q21	27	22 3704	21 4658	0 8120	0 8134	0 000077	4 2593	4 5454	0 1735	-0 3019	0 087675
LSI 5q31	26	22 9231	22 2996	0 7623	0 8153	0 000154	4 2692	5 0482	0 0005	-0 0173	0 918489
CEP 3	26	21 1154	24 0671	0 6485	0 7618	0 000377	3 9231	6 5600	0 0010	0 0253	0 880460
CEP 10	25	17 1600	19 9827	0 6154	0 7148	0 000645	3 7000	2 9155	0 0091	-0 0676	0 723951
CEP 11	26	18 9231	21 5108	0 5655	0 6818	0 000770	3 4231	3 1135	0 0743	-0 1998	0 248755
CEP 8	27	17 2222	21 7155	0 5567	0 7125	0 000646	3 2593	2 9819	0 0863	-0 2077	0 278498
CEP 18	25	17 0000	21 1325	0 5128	0 6574	0 001526	5 0800	4 1122	0 2510	-0 3545	0 070839
LSI 13	27	13 4444	15 3230	0 5040	0 6259	0 001103	4 0741	3 4744	0 0091	0 0677	0 705658
LSI 9p21	26	14 9615	17 5191	0 4736	0 6004	0 001833	9 0000	15 5486	0 0997	0 2693	0 128341
LSI 10q23	25	15 8600	13 9280	0 4520	0 5323	0 003606	3 3600	4 4989	0 0052	0 0541	0 752077
CEP 12	26	19 3462	26 9250	0 4383	0 6330	0 002417	3 2308	3 4212	0 0734	-0 1931	0 286454
CEP 17	27	16 3704	21 9057	0 4065	0 5726	0 002832	6 2222	5 8001	0 4089	-0 4560	0 016682
LSI 17p13	27	14 1852	15 7774	0 3844	0 5111	0 004264	7 6296	9 9466	0 0049	0 0553	0 738974
LSI 21	26	17 7844	17 8255	0 2805	0 4198	0 016950	4 5832	3 8505	0 0622	-0 1777	0 384519
Ratios											
5 p/q imbal	26	28 1566	22 1019	1 0505	0 8962	0 000020	5 3885	7 0458	0 0897	0 2470	0 154145
LSI 7p12/CEP 7	26	15 8237	10 9781	0 6764	0 6496	0 000446	3 8921	4 6890	0 2832	0 4018	0 022057
LSI 8q24/CEP 8	27	13 7445	8 6033	0 6747	0 6340	0 000477	5 2233	7 7125	0 0825	0 2408	0 160626
LSI 3p14/CEP 3	26	12 5385	9 8599	0 2517	0 3736	0 033580	14 6154	18 1242	0 3618	0 5307	0 005431
LSI 17q21/CEP	27	16 7089	9 9709	0 2181	0 3440	0 048699	8 4901	8 3931	0 0699	0 2172	0 200321
LSI 10q23/CEP	25	12 3232	8 1702	0 1431	0 2708	0 138690	11 8277	17 5057	0 2519	0 4596	0 019653
LSI 9p21/CEP 9	26	9 2480	9 1323	0 0171	0 0930	0 608094	13 3700	16 2368	0 3208	0 4688	0 009422
LSI 17p13/CEP	27	11 3749	9 4349	0 0000	0 0051	0 976201	17 4138	20 1651	0 3132	0 4915	0 007889

Table 5 Sensitivity and Specificity of Lung Tumor Detection

CUTOFF = 5% CELLS WITH GAINS OR LOSSES							CUTOFF = 10% CELLS WITH GAINS OR LOSSES						
PROBE	LOSS/GAIN	SPECIFICITY	SENSITIVITY	SENS*SPEC	VECTOR	# TUMOR SPECIMENS	PROBE	LOSS/GAI	SPECIFICIT	SENSITIVITY	SENS*SPEC	VECTOR	# TUMOR SPECIMENS
CEP 1	gain	1 000	0 923	0 923	0 077	26	8q24	gain	1 000	0 778	0 778	0 222	27
8q24	gain	0 900	0 815	0 733	0 210	27	LSI 5p15	gain	1 000	0 769	0 769	0 231	26
CEP 16	gain	1 000	0 737	0 737	0 263	19	7p12	gain	0 900	0 692	0 623	0 324	26
CEP 6	gain	0 900	0 731	0 658	0 287	26	CEP 1	gain	1 000	0 654	0 654	0 346	26
CEP 9	gain	0 900	0 731	0 658	0 287	26	CEP 9	gain	1 000	0 654	0 654	0 346	26
LSI 5q31	gain	0 900	0 692	0 623	0 324	26	LSI 3q	gain	0 900	0 632	0 568	0 382	19
LSI 20q	gain	0 800	0 737	0 589	0 331	19	CEP 6	gain	1 000	0 615	0 615	0 385	26
3p14	gain	0 700	0 846	0 592	0 337	26	17q21	gain	1 000	0 593	0 593	0 407	27
17q21	gain	0 800	0 704	0 563	0 357	27	CEP 16	gain	1 000	0 579	0 579	0 421	19
CEP 4	gain	0 800	0 684	0 547	0 374	19	CEP 4	gain	1 000	0 579	0 579	0 421	19
LSI 5p15	gain	0 600	0 923	0 554	0 407	26	LSI 20q	gain	1 000	0 579	0 579	0 421	19
CEP 8	gain	1 000	0 593	0 593	0 407	27	LSI 5q31	gain	1 000	0 577	0 577	0 423	26
LSI 13	gain	0 900	0 593	0 533	0 420	27	3p14	gain	0 900	0 577	0 519	0 435	26
CEP 11	gain	0 900	0 577	0 519	0 435	26	CEP 7	gain	1 000	0 538	0 538	0 462	26
CEP 10	gain	1 000	0 560	0 560	0 440	25	CEP 3	gain	1 000	0 500	0 500	0 500	26
CEP 17	gain	0 900	0 556	0 500	0 456	27	CEP 8	gain	1 000	0 481	0 481	0 519	27
CEP 3	gain	1 000	0 538	0 538	0 462	26	9p21	gain	1 000	0 462	0 462	0 538	26
CEP 7	gain	1 000	0 538	0 538	0 462	26	CEP 11	gain	1 000	0 462	0 462	0 538	26
9p21	gain	0 800	0 577	0 462	0 468	26	10q23	gain	0 800	0 480	0 384	0 557	25
10q23	gain	0 600	0 720	0 432	0 488	25	CEP 12	gain	1 000	0 423	0 423	0 577	26
CEP 18	gain	0 900	0 520	0 468	0 490	25	LSI 21	gain	0 700	0 500	0 350	0 583	26
CEP 12	gain	1 000	0 500	0 500	0 500	26	CEP 17	gain	1 000	0 407	0 407	0 593	27
17p13	gain	0 600	0 593	0 356	0 571	27	LSI 13	gain	1 000	0 407	0 407	0 593	27
LSI 21	gain	0 500	0 692	0 346	0 587	26	CEP 10	gain	1 000	0 400	0 400	0 600	25
7p12	gain	0 400	0 846	0 338	0 619	26	CEP 18	gain	1 000	0 400	0 400	0 600	25
9p21	loss	0 800	0 385	0 308	0 647	26	17p13	gain	0 900	0 407	0 367	0 601	27
LSI 13	loss	0 700	0 370	0 259	0 697	27	17p13	loss	0 800	0 259	0 207	0 767	27
CEP 1	loss	0 800	0 308	0 246	0 721	26	CEP 9	loss	0 800	0 192	0 154	0 832	26
LSI 3q	gain	0 300	0 789	0 237	0 731	19	LSI 5q31	loss	1 000	0 154	0 154	0 846	26
10q23	loss	0 900	0 240	0 216	0 767	25	9p21	loss	0 900	0 154	0 138	0 852	26
3p14	loss	0 900	0 231	0 208	0 776	26	3p14	loss	1 000	0 077	0 077	0 923	26
CEP 11	loss	0 700	0 269	0 188	0 790	26	CEP 3	loss	1 000	0 077	0 077	0 923	26
CEP 6	loss	0 700	0 269	0 188	0 790	26	CEP 17	loss	0 500	0 222	0 111	0 925	27
CEP 12	loss	0 900	0 192	0 173	0 814	26	LSI 21	loss	0 900	0 077	0 069	0 928	26
CEP 7	loss	0 900	0 192	0 173	0 814	26	17q21	loss	0 900	0 074	0 067	0 931	27
CEP 10	loss	0 700	0 240	0 168	0 817	25	CEP 18	loss	0 800	0 080	0 064	0 941	25
LSI 21	loss	0 500	0 346	0 173	0 823	26	LSI 3q	loss	1 000	0 053	0 053	0 947	19
17q21	loss	0 500	0 333	0 167	0 833	27	CEP 16	loss	0 400	0 263	0 105	0 950	19
CEP 4	loss	1 000	0 158	0 158	0 842	19	10q23	loss	1 000	0 040	0 040	0 960	25
CEP 16	loss	0 300	0 526	0 158	0 845	19	CEP 10	loss	1 000	0 040	0 040	0 960	25
LSI 5q31	loss	0 600	0 231	0 138	0 867	26	CEP 1	loss	1 000	0 038	0 038	0 962	26
CEP 8	loss	0 700	0 185	0 130	0 868	27	CEP 11	loss	1 000	0 038	0 038	0 962	26
CEP 3	loss	0 800	0 154	0 123	0 869	26	CEP 6	loss	1 000	0 038	0 038	0 962	26
LSI 3q	loss	0 800	0 105	0 084	0 917	19	LSI 13	loss	1 000	0 037	0 037	0 963	27
7p12	loss	0 900	0 077	0 069	0 928	26	CEP 12	loss	0 900	0 038	0 035	0 967	26
17p13	loss	0 300	0 370	0 111	0 942	27	7p12	loss	1 000	0 000	0 000	1 000	26
LSI 5p15	loss	0 900	0 038	0 035	0 967	26	8q24	loss	1 000	0 000	0 000	1 000	27
8q24	loss	0 900	0 037	0 033	0 968	27	CEP 4	loss	1 000	0 000	0 000	1 000	19
LSI 20q	loss	0 800	0 053	0 042	0 968	19	CEP 7	loss	1 000	0 000	0 000	1 000	26
CEP 18	loss	0 200	0 400	0 080	1 000	25	CEP 8	loss	1 000	0 000	0 000	1 000	27
CEP 9	loss	0 200	0 385	0 077	1 009	26	LSI 5p15	loss	1 000	0 000	0 000	1 000	26
CEP 17	loss	0 100	0 519	0 052	1 021	27	LSI 20q	loss	0 900	0 000	0 000	1 005	19
ratios:													
5 p/q imbal.	gain	0 600	0 923	0 554	0 407	26	5 p/q imbal	gain	0 800	0 692	0 554	0 367	26
8q24/CEP 8	gain	0 600	0 852	0 511	0 427	27	7p12/CEP 7	gain	0 900	0 577	0 519	0 435	26
3p14/CEP 3	loss	0 700	0 654	0 458	0 458	26	8q24/CEP 8	gain	0 800	0 593	0 474	0 454	27
9p21/CEP 9	loss	0 800	0 577	0 462	0 468	26	3p14/CEP 3	gain	0 900	0 500	0 450	0 510	26
10q23/CEP 1	loss	0 900	0 520	0 468	0 490	25	3p14/CEP 3	loss	1 000	0 462	0 462	0 538	26
7p12/CEP 7	gain	0 500	0 808	0 404	0 536	26	10q23/CEP 1	gain	0 700	0 480	0 336	0 600	25
10q23/CEP 1	gain	0 500	0 760	0 380	0 555	25	17q21/CEP 1	gain	0 500	0 667	0 333	0 601	27
3p14/CEP 3	gain	0 400	0 808	0 323	0 630	26	17p13/CEP 1	loss	0 900	0 407	0 367	0 601	27
8q24/CEP 8	loss	1 000	0 333	0 333	0 667	27	9p21/CEP 9	loss	0 900	0 346	0 312	0 661	26
9p21/CEP 9	gain	0 400	0 615	0 246	0 713	26	17q21/CEP 1	loss	1 000	0 333	0 333	0 667	27
7p12/CEP 7	loss	0 900	0 269	0 242	0 738	26	17p13/CEP 1	gain	0 600	0 407	0 244	0 715	27
17p13/CEP 1	loss	0 300	0 667	0 200	0 775	27	10q23/CEP 1	loss	1 000	0 240	0 240	0 760	25
5 p/q imbal.	loss	0 900	0 231	0 208	0 776	26	9p21/CEP 9	gain	0 800	0 231	0 185	0 795	26
17q21/CEP 1	loss	0 300	0 593	0 178	0 810	27	7p12/CEP 7	loss	1 000	0 192	0 192	0 808	26
17q21/CEP 1	gain	0 100	0 889	0 089	0 907	27	8q24/CEP 8	loss	1 000	0 111	0 111	0 889	27
17p13/CEP 1	gain	0 100	0 704	0 070	0 948	27	5 p/q imbal	loss	1 000	0 038	0 038	0 962	26

CUTOFF = 20% CELLS WITH GAINS OR LOSSES							CUTOFF = 30% CELLS WITH GAINS OR LOSSES						
PROBE	LOSS/GAIN	SPECIFICITY	SENSITIVITY	SENS*SPEC	VECTOR	# TUMOR SPECIMENS	PROBE	LOSS/GAI	SPECIFICIT	SENSITIVITY	SENS*SPEC	VECTOR	# TUMOR SPECIMENS
LSI 5p15	gain	1 000	0 654	0 654	0 346	26	LSI 5p15	gain	1 000	0 577	0 577	0 423	26
7p12	gain	1 000	0 615	0 615	0 385	26	7p12	gain	1 000	0 500	0 500	0 500	26
LSI 3q	gain	1 000	0 579	0 579	0 421	19	CEP 6	gain	1 000	0 500	0 500	0 500	26
CEP 1	gain	1 000	0 538	0 538	0 462	26	LSI 20q	gain	1 000	0 474	0 474	0 526	19
3p14	gain	1 000	0 500	0 500	0 500	26	LSI 3q	gain	1 000	0 474	0 474	0 526	19
CEP 6	gain	1 000	0 500	0 500	0 500	26	CEP 1	gain	1 000	0 385	0 385	0 615	26

CEP 16	gain	1 000	0 474	0 474	0 526	19	CEP 7	gain	1 000	0 385	0 385	0 615	26
CEP 4	gain	1 000	0 474	0 474	0 526	19	3p14	gain	1 000	0 346	0 346	0 654	26
LSI 20q	gain	1 000	0 474	0 474	0 526	19	LSI 5q31	gain	1 000	0 346	0 346	0 654	26
CEP 7	gain	1 000	0 462	0 462	0 538	26	CEP 3	gain	1 000	0 308	0 308	0 692	26
17q21	gain	1 000	0 444	0 444	0 556	27	17q21	gain	1 000	0 296	0 296	0 704	27
8q24	gain	1 000	0 444	0 444	0 556	27	CEP 11	gain	1 000	0 269	0 269	0 731	26
CEP 3	gain	1 000	0 423	0 423	0 577	26	CEP 12	gain	1 000	0 269	0 269	0 731	26
CEP 9	gain	1 000	0 423	0 423	0 577	26	CEP 16	gain	1 000	0 263	0 263	0 737	19
LSI 5q31	gain	1 000	0 423	0 423	0 577	26	CEP 4	gain	1 000	0 263	0 263	0 737	19
CEP 11	gain	1 000	0 385	0 385	0 615	26	CEP 10	gain	1 000	0 240	0 240	0 760	25
CEP 10	gain	1 000	0 360	0 360	0 640	25	CEP 18	gain	1 000	0 240	0 240	0 760	25
CEP 12	gain	1 000	0 346	0 346	0 654	26	8q24	gain	1 000	0 222	0 222	0 778	27
10q23	gain	1 000	0 320	0 320	0 680	25	CEP 17	gain	1 000	0 222	0 222	0 778	27
CEP 18	gain	1 000	0 320	0 320	0 680	25	CEP 9	gain	1 000	0 192	0 192	0 808	26
CEP 17	gain	1 000	0 296	0 296	0 704	27	LSI 21	gain	1 000	0 192	0 192	0 808	26
CEP 8	gain	1 000	0 296	0 296	0 704	27	17p13	gain	1 000	0 185	0 185	0 815	27
LSI 21	gain	0 900	0 269	0 242	0 738	26	CEP 8	gain	1 000	0 185	0 185	0 815	27
9p21	gain	1 000	0 231	0 231	0 769	26	10q23	gain	1 000	0 160	0 160	0 840	25
17p13	gain	1 000	0 222	0 222	0 778	27	9p21	gain	1 000	0 154	0 154	0 846	26
LSI 13	gain	1 000	0 148	0 148	0 852	27	9p21	loss	1 000	0 115	0 115	0 885	26
9p21	loss	1 000	0 115	0 115	0 885	26	LSI 13	gain	1 000	0 111	0 111	0 889	27
CEP 3	loss	1 000	0 077	0 077	0 923	26	CEP 1	loss	1 000	0 038	0 038	0 962	26
17p13	loss	1 000	0 074	0 074	0 926	27	CEP 9	loss	1 000	0 038	0 038	0 962	26
CEP 16	loss	1 000	0 053	0 053	0 947	19	17p13	loss	1 000	0 037	0 037	0 963	27
LSI 3q	loss	1 000	0 053	0 053	0 947	19	10q23	loss	1 000	0 000	0 000	1 000	25
3p14	loss	1 000	0 038	0 038	0 962	26	17q21	loss	1 000	0 000	0 000	1 000	27
CEP 1	loss	1 000	0 038	0 038	0 962	26	3p14	loss	1 000	0 000	0 000	1 000	26
CEP 9	loss	1 000	0 038	0 038	0 962	26	7p12	loss	1 000	0 000	0 000	1 000	26
LSI 5q31	loss	1 000	0 038	0 038	0 962	26	8q24	loss	1 000	0 000	0 000	1 000	27
CEP 17	loss	1 000	0 037	0 037	0 963	27	CEP 10	loss	1 000	0 000	0 000	1 000	25
10q23	loss	1 000	0 000	0 000	1 000	25	CEP 11	loss	1 000	0 000	0 000	1 000	26
17q21	loss	1 000	0 000	0 000	1 000	27	CEP 12	loss	1 000	0 000	0 000	1 000	26
7p12	loss	1 000	0 000	0 000	1 000	26	CEP 16	loss	1 000	0 000	0 000	1 000	19
8q24	loss	1 000	0 000	0 000	1 000	27	CEP 17	loss	1 000	0 000	0 000	1 000	27
CEP 10	loss	1 000	0 000	0 000	1 000	25	CEP 18	loss	1 000	0 000	0 000	1 000	25
CEP 11	loss	1 000	0 000	0 000	1 000	26	CEP 3	loss	1 000	0 000	0 000	1 000	26
CEP 12	loss	1 000	0 000	0 000	1 000	26	CEP 4	loss	1 000	0 000	0 000	1 000	19
CEP 18	loss	1 000	0 000	0 000	1 000	25	CEP 6	loss	1 000	0 000	0 000	1 000	26
CEP 4	loss	1 000	0 000	0 000	1 000	19	CEP 7	loss	1 000	0 000	0 000	1 000	26
CEP 6	loss	1 000	0 000	0 000	1 000	26	CEP 8	loss	1 000	0 000	0 000	1 000	27
CEP 7	loss	1 000	0 000	0 000	1 000	26	LSI 13	loss	1 000	0 000	0 000	1 000	27
CEP 8	loss	1 000	0 000	0 000	1 000	27	LSI 20q	loss	1 000	0 000	0 000	1 000	19
LSI 13	loss	1 000	0 000	0 000	1 000	27	LSI 21	loss	1 000	0 000	0 000	1 000	26
LSI 20q	loss	1 000	0 000	0 000	1 000	19	LSI 3q	loss	1 000	0 000	0 000	1 000	19
LSI 21	loss	1 000	0 000	0 000	1 000	26	LSI 5p15	loss	1 000	0 000	0 000	1 000	26
LSI 5p15	loss	1 000	0 000	0 000	1 000	26	LSI 5q31	loss	1 000	0 000	0 000	1 000	26
5 p/q imbal.	gain	1 000	0 500	0 500	0 500	26	5 p/q imbal	gain	1 000	0 385	0 385	0 615	26
17q21/CEP 1	gain	1 000	0 370	0 370	0 630	27	17p13/CEP 1	loss	1 000	0 185	0 185	0 815	27
7p12/CEP 7	gain	1 000	0 346	0 346	0 654	26	10q23/CEP 1	loss	1 000	0 160	0 160	0 840	25
17p13/CEP 1	loss	1 000	0 259	0 259	0 741	27	3p14/CEP 3	loss	1 000	0 115	0 115	0 885	26
3p14/CEP 3	loss	1 000	0 192	0 192	0 808	26	3p14/CEP 3	gain	1 000	0 115	0 115	0 885	26
9p21/CEP 9	loss	1 000	0 192	0 192	0 808	26	7p12/CEP 7	gain	1 000	0 115	0 115	0 885	26
17p13/CEP 1	gain	0 900	0 185	0 167	0 821	27	9p21/CEP 9	loss	1 000	0 115	0 115	0 885	26
10q23/CEP 1	loss	1 000	0 160	0 160	0 840	25	10q23/CEP 1	gain	1 000	0 080	0 080	0 920	25
3p14/CEP 3	gain	1 000	0 154	0 154	0 846	26	9p21/CEP 9	gain	1 000	0 077	0 077	0 923	26
8q24/CEP 8	gain	1 000	0 148	0 148	0 852	27	17q21/CEP 1	gain	1 000	0 074	0 074	0 926	27
10q23/CEP 1	gain	1 000	0 120	0 120	0 880	25	17p13/CEP 1	gain	1 000	0 037	0 037	0 963	27
17q21/CEP 1	loss	1 000	0 111	0 111	0 889	27	17q21/CEP 1	loss	1 000	0 037	0 037	0 963	27
9p21/CEP 9	gain	0 900	0 077	0 069	0 928	26	8q24/CEP 8	loss	1 000	0 037	0 037	0 963	27
8q24/CEP 8	loss	1 000	0 037	0 037	0 963	27	8q24/CEP 8	gain	1 000	0 037	0 037	0 963	27
5 p/q imbal.	loss	1 000	0 000	0 000	1 000	26	5 p/q imbal	loss	1 000	0 000	0 000	1 000	26
7p12/CEP 7	loss	1 000	0 000	0 000	1 000	26	7p12/CEP 7	loss	1 000	0 000	0 000	1 000	26

CUTOFF = 40% CELLS WITH GAINS OR LOSSES							CUTOFF = 50% CELLS WITH GAINS OR LOSSES						
PROBE	LOSS/GAIN	SPECIFICITY	SENSITIVITY	SENS*SPEC	VECTOR	# TUMOR SPECIMENS	PROBE	LOSS/GAI	SPECIFICIT	SENSITIVITY	SENS*SPEC	VECTOR	# TUMOR SPECIMENS
7p12	gain	1 000	0 385	0 385	0 615	26	CEP 1	gain	1 000	0 231	0 231	0 769	26
CEP 1	gain	1 000	0 346	0 346	0 654	26	LSI 20q	gain	1 000	0 211	0 211	0 789	19
LSI 3q	gain	1 000	0 316	0 316	0 684	19	LSI 3q	gain	1 000	0 211	0 211	0 789	19
CEP 6	gain	1 000	0 308	0 308	0 692	26	CEP 7	gain	1 000	0 192	0 192	0 808	26
CEP 7	gain	1 000	0 308	0 308	0 692	26	LSI 5p15	gain	1 000	0 192	0 192	0 808	26
LSI 5p15	gain	1 000	0 308	0 308	0 692	26	7p12	gain	1 000	0 154	0 154	0 846	26
LSI 20q	gain	1 000	0 211	0 211	0 789	19	CEP 11	gain	1 000	0 154	0 154	0 846	26
CEP 18	gain	1 000	0 200	0 200	0 800	25	CEP 3	gain	1 000	0 154	0 154	0 846	26
17q21	gain	1 000	0 185	0 185	0 815	27	CEP 6	gain	1 000	0 154	0 154	0 846	26
CEP 10	gain	1 000	0 160	0 160	0 840	25	CEP 12	gain	1 000	0 115	0 115	0 885	26
CEP 16	gain	1 000	0 158	0 158	0 842	19	LSI 5q31	gain	1 000	0 115	0 115	0 885	26
CEP 4	gain	1 000	0 158	0 158	0 842	19	17q21	gain	1 000	0 111	0 111	0 889	27
CEP 11	gain	1 000	0 154	0 154	0 846	26	8q24	gain	1 000	0 111	0 111	0 889	27
CEP 12	gain	1 000	0 154	0 154	0 846	26	CEP 17	gain	1 000	0 111	0 111	0 889	27
CEP 3	gain	1 000	0 154	0 154	0 846	26	CEP 8	gain	1 000	0 111	0 111	0 889	27
CEP 17	gain	1 000	0 148	0 148	0 852	27	CEP 16	gain	1 000	0 105	0 105	0 895	19
3p14	gain	1 000	0 115	0 115	0 885	26	CEP 4	gain	1 000	0 105	0 105	0 895	19
9p21	loss	1 000	0 115	0 115	0 885	26	CEP 10	gain	1 000	0 080	0 080	0 920	25
CEP 9	gain	1 000	0 115	0 115	0 885	26	CEP 18	gain	1 000	0 080	0 080	0 920	25

LSI 21	gain	1 000	0 115	0 115	0 885	26	3p14	gain	1 000	0 077	0 077	0 923	26
LSI 5q31	gain	1 000	0 115	0 115	0 885	26	LSI 21	gain	1 000	0 077	0 077	0 923	26
17p13	gain	1 000	0 111	0 111	0 889	27	17p13	gain	1 000	0 074	0 074	0 926	27
8q24	gain	1 000	0 111	0 111	0 889	27	9p21	loss	1 000	0 038	0 038	0 962	26
CEP 8	gain	1 000	0 111	0 111	0 889	27	9p21	gain	1 000	0 038	0 038	0 962	26
10q23	gain	1 000	0 040	0 040	0 960	25	CEP 1	loss	1 000	0 038	0 038	0 962	26
9p21	gain	1 000	0 038	0 038	0 962	26	CEP 9	gain	1 000	0 038	0 038	0 962	26
CEP 1	loss	1 000	0 038	0 038	0 962	26	LSI 13	gain	1 000	0 037	0 037	0 963	27
CEP 9	loss	1 000	0 038	0 038	0 962	26	10q23	loss	1 000	0 000	0 000	1 000	25
17p13	loss	1 000	0 037	0 037	0 963	27	10q23	gain	1 000	0 000	0 000	1 000	25
LSI 13	gain	1 000	0 037	0 037	0 963	27	17p13	loss	1 000	0 000	0 000	1 000	27
10q23	loss	1 000	0 000	0 000	1 000	25	17q21	loss	1 000	0 000	0 000	1 000	27
17q21	loss	1 000	0 000	0 000	1 000	27	3p14	loss	1 000	0 000	0 000	1 000	26
3p14	loss	1 000	0 000	0 000	1 000	26	7p12	loss	1 000	0 000	0 000	1 000	26
7p12	loss	1 000	0 000	0 000	1 000	26	8q24	loss	1 000	0 000	0 000	1 000	27
8q24	loss	1 000	0 000	0 000	1 000	27	CEP 10	loss	1 000	0 000	0 000	1 000	25
CEP 10	loss	1 000	0 000	0 000	1 000	25	CEP 11	loss	1 000	0 000	0 000	1 000	26
CEP 11	loss	1 000	0 000	0 000	1 000	26	CEP 12	loss	1 000	0 000	0 000	1 000	26
CEP 12	loss	1 000	0 000	0 000	1 000	26	CEP 16	loss	1 000	0 000	0 000	1 000	19
CEP 16	loss	1 000	0 000	0 000	1 000	19	CEP 17	loss	1 000	0 000	0 000	1 000	27
CEP 17	loss	1 000	0 000	0 000	1 000	27	CEP 18	loss	1 000	0 000	0 000	1 000	25
CEP 18	loss	1 000	0 000	0 000	1 000	25	CEP 3	loss	1 000	0 000	0 000	1 000	26
CEP 3	loss	1 000	0 000	0 000	1 000	26	CEP 4	loss	1 000	0 000	0 000	1 000	19
CEP 4	loss	1 000	0 000	0 000	1 000	19	CEP 6	loss	1 000	0 000	0 000	1 000	26
CEP 6	loss	1 000	0 000	0 000	1 000	26	CEP 7	loss	1 000	0 000	0 000	1 000	26
CEP 7	loss	1 000	0 000	0 000	1 000	26	CEP 8	loss	1 000	0 000	0 000	1 000	27
CEP 8	loss	1 000	0 000	0 000	1 000	27	CEP 9	loss	1 000	0 000	0 000	1 000	26
LSI 13	loss	1 000	0 000	0 000	1 000	27	LSI 13	loss	1 000	0 000	0 000	1 000	27
LSI 20q	loss	1 000	0 000	0 000	1 000	19	LSI 20q	loss	1 000	0 000	0 000	1 000	19
LSI 21	loss	1 000	0 000	0 000	1 000	26	LSI 21	loss	1 000	0 000	0 000	1 000	26
LSI 3q	loss	1 000	0 000	0 000	1 000	19	LSI 3q	loss	1 000	0 000	0 000	1 000	19
LSI 5p15	loss	1 000	0 000	0 000	1 000	26	LSI 5p15	loss	1 000	0 000	0 000	1 000	26
LSI 5q31	loss	1 000	0 000	0 000	1 000	26	LSI 5q31	loss	1 000	0 000	0 000	1 000	26
5 p/q imbal.	gain	1 000	0 192	0 192	0 808	26	5 p/q imbal	gain	1 000	0 115	0 115	0 885	26
17p13/CEP 1	loss	1 000	0 185	0 185	0 815	27	17p13/CEP 1	loss	1 000	0 111	0 111	0 889	27
10q23/CEP 1	loss	1 000	0 080	0 080	0 920	25	10q23/CEP 1	loss	1 000	0 080	0 080	0 920	25
3p14/CEP 3	loss	1 000	0 077	0 077	0 923	26	3p14/CEP 3	loss	1 000	0 077	0 077	0 923	26
9p21/CEP 9	loss	1 000	0 077	0 077	0 923	26	9p21/CEP 9	loss	1 000	0 077	0 077	0 923	26
9p21/CEP 9	gain	1 000	0 038	0 038	0 962	26	5 p/q imbal	loss	1 000	0 000	0 000	1 000	26
5 p/q imbal.	loss	1 000	0 000	0 000	1 000	26	10q23/CEP 1	gain	1 000	0 000	0 000	1 000	25
10q23/CEP 1	gain	1 000	0 000	0 000	1 000	25	17p13/CEP 1	gain	1 000	0 000	0 000	1 000	27
17p13/CEP 1	gain	1 000	0 000	0 000	1 000	27	17q21/CEP 1	loss	1 000	0 000	0 000	1 000	27
17q21/CEP 1	loss	1 000	0 000	0 000	1 000	27	17q21/CEP 1	gain	1 000	0 000	0 000	1 000	27
17q21/CEP 1	gain	1 000	0 000	0 000	1 000	27	3p14/CEP 3	gain	1 000	0 000	0 000	1 000	26
3p14/CEP 3	gain	1 000	0 000	0 000	1 000	26	7p12/CEP 7	loss	1 000	0 000	0 000	1 000	26
7p12/CEP 7	loss	1 000	0 000	0 000	1 000	26	7p12/CEP 7	gain	1 000	0 000	0 000	1 000	26
7p12/CEP 7	gain	1 000	0 000	0 000	1 000	26	8q24/CEP 8	loss	1 000	0 000	0 000	1 000	27
8q24/CEP 8	loss	1 000	0 000	0 000	1 000	27	8q24/CEP 8	gain	1 000	0 000	0 000	1 000	27
8q24/CEP 8	gain	1 000	0 000	0 000	1 000	27	9p21/CEP 9	gain	1 000	0 000	0 000	1 000	26

Table 6 Combinations of 2, 3 and 4 Probes at a Cutoff Value of 10%

PROBE 1		PROBE 2		PROBE 3		PROBE 4	SPECIFICITY	SENSITIVITY	SENS*SPEC	VECTOR	# TUMOR SPECIMENS
2 probe combinations											
CEP 17	gain	8q24	gain				1 000	0 852	0 852	0 148	27
8q24	gain	CEP 1	gain				1 000	0 846	0 846	0 154	26
8q24	gain	LSI 5p15	gain				1 000	0 846	0 846	0 154	26
CEP 12	gain	LSI 5p15	gain				1 000	0 846	0 846	0 154	26
17q21	gain	8q24	gain				1 000	0 815	0 815	0 185	27
17q21	gain	CEP 1	gain				1 000	0 808	0 808	0 192	26
17q21	gain	LSI 5p15	gain				1 000	0 808	0 808	0 192	26
8q24	gain	CEP 6	gain				1 000	0 808	0 808	0 192	26
8q24	gain	CEP 7	gain				1 000	0 808	0 808	0 192	26
8q24	gain	LSI 5q31	gain				1 000	0 808	0 808	0 192	26
9p21	gain	8q24	gain				1 000	0 808	0 808	0 192	26
9p21	gain	LSI 5p15	gain				1 000	0 808	0 808	0 192	26
CEP 11	gain	8q24	gain				1 000	0 808	0 808	0 192	26
CEP 17	gain	LSI 5p15	gain				1 000	0 808	0 808	0 192	26
CEP 8	gain	LSI 5p15	gain				1 000	0 808	0 808	0 192	26
CEP 9	gain	8q24	gain				1 000	0 808	0 808	0 192	26
LSI 13	gain	LSI 5p15	gain				1 000	0 808	0 808	0 192	26
LSI 5q31	gain	LSI 5p15	gain				1 000	0 808	0 808	0 192	26
LSI 5p15	gain	LSI 3q	gain				0 875	0 842	0 737	0 201	19
17p13	gain	8q24	gain				0 900	0 815	0 733	0 210	27
8q24	gain	CEP 4	gain				1 000	0 789	0 789	0 211	19
CEP 16	gain	8q24	gain				1 000	0 789	0 789	0 211	19
CEP 16	gain	CEP 1	gain				1 000	0 789	0 789	0 211	19
CEP 16	gain	LSI 5p15	gain				1 000	0 789	0 789	0 211	19
CEP 16	gain	LSI 5q31	gain				1 000	0 789	0 789	0 211	19
CEP 17	gain	CEP 16	gain				1 000	0 789	0 789	0 211	19
LSI 20q	gain	8q24	gain				1 000	0 789	0 789	0 211	19
LSI 20q	gain	CEP 1	gain				1 000	0 789	0 789	0 211	19
LSI 20q	gain	LSI 5p15	gain				1 000	0 789	0 789	0 211	19
LSI 5p15	gain	CEP 4	gain				1 000	0 789	0 789	0 211	19
3 probe combinations											
9p21	gain	8q24	gain	LSI 5p15	gain		1 000	0 885	0 885	0 115	26
9p21	gain	8q24	gain	CEP 1	gain		1 000	0 885	0 885	0 115	26
CEP 12	gain	9p21	gain	LSI 5p15	gain		1 000	0 885	0 885	0 115	26
CEP 17	gain	9p21	gain	8q24	gain		1 000	0 885	0 885	0 115	26
17q21	gain	9p21	gain	8q24	gain		1 000	0 846	0 846	0 154	26
17q21	gain	9p21	gain	CEP 8	gain		1 000	0 846	0 846	0 154	26
17q21	gain	9p21	gain	LSI 5p15	gain		1 000	0 846	0 846	0 154	26
17q21	gain	9p21	gain	CEP 1	gain		1 000	0 846	0 846	0 154	26
17q21	gain	CEP 12	gain	CEP 1	gain		1 000	0 846	0 846	0 154	26
17q21	gain	CEP 8	gain	LSI 5p15	gain		1 000	0 846	0 846	0 154	26
17q21	gain	CEP 8	gain	CEP 1	gain		1 000	0 846	0 846	0 154	26
17q21	gain	LSI 13	gain	9p21	gain		1 000	0 846	0 846	0 154	26
17q21	gain	LSI 13	gain	LSI 5p15	gain		1 000	0 846	0 846	0 154	26
17q21	gain	LSI 13	gain	CEP 1	gain		1 000	0 846	0 846	0 154	26
9p21	gain	8q24	gain	CEP 7	gain		1 000	0 846	0 846	0 154	26
9p21	gain	8q24	gain	CEP 6	gain		1 000	0 846	0 846	0 154	26
9p21	gain	8q24	gain	LSI 5q31	gain		1 000	0 846	0 846	0 154	26
9p21	gain	CEP 8	gain	LSI 5p15	gain		1 000	0 846	0 846	0 154	26
9p21	gain	CEP 8	gain	CEP 1	gain		1 000	0 846	0 846	0 154	26
9p21	gain	CEP 9	gain	8q24	gain		1 000	0 846	0 846	0 154	26
9p21	gain	LSI 5q31	gain	LSI 5p15	gain		1 000	0 846	0 846	0 154	26
CEP 11	gain	9p21	gain	8q24	gain		1 000	0 846	0 846	0 154	26
CEP 12	gain	9p21	gain	CEP 6	gain		1 000	0 846	0 846	0 154	26
CEP 12	gain	CEP 6	gain	CEP 1	gain		1 000	0 846	0 846	0 154	26
CEP 17	gain	9p21	gain	LSI 5p15	gain		1 000	0 846	0 846	0 154	26
CEP 17	gain	CEP 8	gain	LSI 5p15	gain		1 000	0 846	0 846	0 154	26
CEP 17	gain	CEP 9	gain	CEP 8	gain		1 000	0 846	0 846	0 154	26

CEP 17	gain	CEP 9	gain	CEP 8	gain			1 000	0 846	0 846	0 154	26
CEP 17	gain	LSI 13	gain	CEP 9	gain			1 000	0 846	0 846	0 154	26
CEP 17	gain	LSI 13	gain	LSI 5p15	gain			1 000	0 846	0 846	0 154	26
CEP 8	gain	LSI 5q31	gain	LSI 5p15	gain			1 000	0 846	0 846	0 154	26
CEP 8	gain	LSI 5q31	gain	CEP 1	gain			1 000	0 846	0 846	0 154	26
LSI 13	gain	9p21	gain	LSI 5p15	gain			1 000	0 846	0 846	0 154	26
LSI 13	gain	9p21	gain	CEP 1	gain			1 000	0 846	0 846	0 154	26
LSI 13	gain	LSI 5q31	gain	LSI 5p15	gain			1 000	0 846	0 846	0 154	26
LSI 13	gain	LSI 5q31	gain	CEP 1	gain			1 000	0 846	0 846	0 154	26
4 probe combinations												
17q21	gain	9p21	gain	CEP 8	gain	LSI 5p15	gain	1 000	0 885	0 885	0 115	26
17q21	gain	9p21	gain	CEP 8	gain	CEP 1	gain	1 000	0 885	0 885	0 115	26
17q21	gain	CEP 12	gain	9p21	gain	CEP 1	gain	1 000	0 885	0 885	0 115	26
17q21	gain	CEP 17	gain	LSI 13	gain	9p21	gain	1 000	0 885	0 885	0 115	26
17q21	gain	CEP 17	gain	9p21	gain	CEP 8	gain	1 000	0 885	0 885	0 115	26
17q21	gain	LSI 13	gain	9p21	gain	LSI 5p15	gain	1 000	0 885	0 885	0 115	26
17q21	gain	LSI 13	gain	9p21	gain	CEP 1	gain	1 000	0 885	0 885	0 115	26
9p21	gain	CEP 8	gain	LSI 5q31	gain	LSI 5p15	gain	1 000	0 885	0 885	0 115	26
9p21	gain	CEP 8	gain	LSI 5q31	gain	CEP 1	gain	1 000	0 885	0 885	0 115	26
CEP 12	gain	9p21	gain	CEP 8	gain	CEP 1	gain	1 000	0 885	0 885	0 115	26
CEP 12	gain	9p21	gain	CEP 6	gain	CEP 1	gain	1 000	0 885	0 885	0 115	26
CEP 12	gain	9p21	gain	CEP 3	gain	CEP 1	gain	1 000	0 885	0 885	0 115	26
CEP 17	gain	9p21	gain	CEP 9	gain	CEP 8	gain	1 000	0 885	0 885	0 115	26
CEP 17	gain	9p21	gain	CEP 8	gain	CEP 6	gain	1 000	0 885	0 885	0 115	26
CEP 17	gain	9p21	gain	CEP 8	gain	LSI 5p15	gain	1 000	0 885	0 885	0 115	26
CEP 17	gain	9p21	gain	CEP 8	gain	CEP 1	gain	1 000	0 885	0 885	0 115	26
CEP 17	gain	CEP 12	gain	9p21	gain	CEP 6	gain	1 000	0 885	0 885	0 115	26
CEP 17	gain	LSI 13	gain	9p21	gain	CEP 9	gain	1 000	0 885	0 885	0 115	26
CEP 17	gain	LSI 13	gain	9p21	gain	CEP 6	gain	1 000	0 885	0 885	0 115	26
CEP 17	gain	LSI 13	gain	9p21	gain	LSI 5p15	gain	1 000	0 885	0 885	0 115	26
CEP 17	gain	LSI 13	gain	9p21	gain	CEP 1	gain	1 000	0 885	0 885	0 115	26
LSI 13	gain	9p21	gain	LSI 5q31	gain	LSI 5p15	gain	1 000	0 885	0 885	0 115	26
LSI 13	gain	9p21	gain	LSI 5q31	gain	CEP 1	gain	1 000	0 885	0 885	0 115	26
LSI 13	gain	CEP 12	gain	9p21	gain	CEP 1	gain	1 000	0 885	0 885	0 115	26
CEP 17	gain	CEP 10	gain	9p21	gain	CEP 8	gain	1 000	0 880	0 880	0 120	25
CEP 17	gain	LSI 13	gain	CEP 10	gain	9p21	gain	1 000	0 880	0 880	0 120	25

Table 7 Combinations of 2, 3 and 4 Probes at a Cutoff Value of 20%

PROBE 1		PROBE 2		PROBE 3		PROBE 4		SPECIFICITY	SENSITIVITY	SENS*SPEC	VECTOR	# TUMOR SPECIMENS
2 probe combinations												
LSI 5p15	gain	LSI 3q	gain					1 000	0 789	0 789	0 211	19
CEP 16	gain	LSI 5p15	gain					1 000	0 737	0 737	0 263	19
LSI 20q	gain	LSI 5p15	gain					1 000	0 737	0 737	0 263	19
LSI 5p15	gain	CEP 4	gain					1 000	0 737	0 737	0 263	19
17q21	gain	LSI 5p15	gain					1 000	0 731	0 731	0 269	26
8q24	gain	LSI 5p15	gain					1 000	0 731	0 731	0 269	26
CEP 6	gain	LSI 5p15	gain					1 000	0 731	0 731	0 269	26
CEP 9	gain	LSI 5p15	gain					1 000	0 731	0 731	0 269	26
LSI 5p15	gain	3p14	gain					1 000	0 731	0 731	0 269	26
LSI 5p15	gain	CEP 3	gain					1 000	0 731	0 731	0 269	26
5 p/q imbal.	gain	LSI 5p15	gain					1 000	0 692	0 692	0 308	26
5 p/q imbal.	gain	LSI 5q31	gain					1 000	0 692	0 692	0 308	26
7p12	gain	LSI 5p15	gain					1 000	0 692	0 692	0 308	26
8q24	gain	7p12	gain					1 000	0 692	0 692	0 308	26
8q24	gain	CEP 6	gain					1 000	0 692	0 692	0 308	26
CEP 12	gain	LSI 5p15	gain					1 000	0 692	0 692	0 308	26
CEP 17	gain	LSI 5p15	gain					1 000	0 692	0 692	0 308	26
CEP 7	gain	LSI 5p15	gain					1 000	0 692	0 692	0 308	26
CEP 8	gain	LSI 5p15	gain					1 000	0 692	0 692	0 308	26
CEP 9	gain	3p14	gain					1 000	0 692	0 692	0 308	26
LSI 13	gain	LSI 5p15	gain					1 000	0 692	0 692	0 308	26
LSI 5p15	gain	CEP 1	gain					1 000	0 692	0 692	0 308	26
7p12	gain	LSI 3q	gain					1 000	0 684	0 684	0 316	19
CEP 12	gain	LSI 3q	gain					1 000	0 684	0 684	0 316	19
CEP 7	gain	LSI 3q	gain					1 000	0 684	0 684	0 316	19
LSI 5q31	gain	LSI 3q	gain					1 000	0 684	0 684	0 316	19
3 probe combinations and 3 pr comb (1 rat + 1 abs)												
CEP 12	gain	LSI 5p15	gain	LSI 3q	gain			1 000	0 842	0 842	0 158	19
5 p/q imbal.	gain	LSI 5q31	gain	LSI 3q	gain			1 000	0 789	0 789	0 211	19
8q24	gain	LSI 5p15	gain	CEP 4	gain			1 000	0 789	0 789	0 211	19
CEP 12	gain	LSI 5p15	gain	CEP 4	gain			1 000	0 789	0 789	0 211	19
CEP 16	gain	8q24	gain	LSI 5p15	gain			1 000	0 789	0 789	0 211	19
CEP 16	gain	CEP 12	gain	LSI 5p15	gain			1 000	0 789	0 789	0 211	19
LSI 20q	gain	8q24	gain	LSI 5p15	gain			1 000	0 789	0 789	0 211	19
LSI 20q	gain	CEP 12	gain	LSI 5p15	gain			1 000	0 789	0 789	0 211	19
17q21	gain	5 p/q imbal	gain	LSI 5q31	gain			1 000	0 769	0 769	0 231	26
17q21	gain	8q24	gain	LSI 5p15	gain			1 000	0 769	0 769	0 231	26
17q21	gain	CEP 12	gain	LSI 5p15	gain			1 000	0 769	0 769	0 231	26
17q21	gain	LSI 5p15	gain	3p14	gain			1 000	0 769	0 769	0 231	26
17q21	gain	LSI 5p15	gain	CEP 3	gain			1 000	0 769	0 769	0 231	26
5 p/q imbal.	gain	LSI 5p15	gain	3p14	gain			1 000	0 769	0 769	0 231	26
5 p/q imbal.	gain	LSI 5p15	gain	CEP 3	gain			1 000	0 769	0 769	0 231	26
5 p/q imbal.	gain	LSI 5q31	gain	3p14	gain			1 000	0 769	0 769	0 231	26
5 p/q imbal.	gain	LSI 5q31	gain	CEP 3	gain			1 000	0 769	0 769	0 231	26
8q24	gain	5 p/q imbal	gain	LSI 5q31	gain			1 000	0 769	0 769	0 231	26
8q24	gain	5 p/q imbal	gain	LSI 5p15	gain			1 000	0 769	0 769	0 231	26
8q24	gain	CEP 6	gain	LSI 5p15	gain			1 000	0 769	0 769	0 231	26
8q24	gain	LSI 5p15	gain	3p14	gain			1 000	0 769	0 769	0 231	26
8q24	gain	LSI 5p15	gain	CEP 3	gain			1 000	0 769	0 769	0 231	26
CEP 12	gain	8q24	gain	LSI 5p15	gain			1 000	0 769	0 769	0 231	26
CEP 12	gain	CEP 6	gain	LSI 5p15	gain			1 000	0 769	0 769	0 231	26
CEP 12	gain	CEP 9	gain	LSI 5p15	gain			1 000	0 769	0 769	0 231	26
CEP 12	gain	CEP 9	gain	LSI 5p15	gain			1 000	0 769	0 769	0 231	26
CEP 12	gain	LSI 5p15	gain	3p14	gain			1 000	0 769	0 769	0 231	26
CEP 12	gain	LSI 5p15	gain	CEP 3	gain			1 000	0 769	0 769	0 231	26
CEP 6	gain	5 p/q imbal	gain	LSI 5q31	gain			1 000	0 769	0 769	0 231	26
CEP 6	gain	5 p/q imbal	gain	LSI 5p15	gain			1 000	0 769	0 769	0 231	26
CEP 6	gain	LSI 5p15	gain	3p14	gain			1 000	0 769	0 769	0 231	26

CEP 6	gain	LSI 5p15	gain	CEP 3	gain			1 000	0 769	0 769	0 231	26
CEP 9	gain	5 p/q imbal	gain	LSI 5q31	gain			1 000	0 769	0 769	0 231	26
CEP 9	gain	5 p/q imbal	gain	LSI 5p15	gain			1 000	0 769	0 769	0 231	26
CEP 9	gain	8q24	gain	3p14	gain			1 000	0 769	0 769	0 231	26
CEP 9	gain	LSI 5p15	gain	3p14	gain			1 000	0 769	0 769	0 231	26
CEP 9	gain	LSI 5p15	gain	CEP 3	gain			1 000	0 769	0 769	0 231	26
4 probe combinations and 4 pr comb (1 rat + 2 abs)												
CEP 12	gain	8q24	gain	LSI 5p15	gain	CEP 4	gain	1 000	0 842	0 842	0 158	19
CEP 12	gain	5 p/q imbal	gain	LSI 5q31	gain	LSI 3q	gain	1 000	0 842	0 842	0 158	19
CEP 16	gain	CEP 12	gain	8q24	gain	LSI 5p15	gain	1 000	0 842	0 842	0 158	19
LSI 20q	gain	CEP 12	gain	8q24	gain	LSI 5p15	gain	1 000	0 842	0 842	0 158	19
17p13	gain	CEP 9	gain	5 p/q imbal	gain	CEP 3	gain	1 000	0 808	0 808	0 192	26
17q21	gain	CEP 12	gain	5 p/q imbal	gain	LSI 5q31	gain	1 000	0 808	0 808	0 192	26
17q21	gain	CEP 12	gain	8q24	gain	LSI 5p15	gain	1 000	0 808	0 808	0 192	26
17q21	gain	CEP 12	gain	LSI 5p15	gain	3p14	gain	1 000	0 808	0 808	0 192	26
17q21	gain	CEP 12	gain	LSI 5p15	gain	CEP 3	gain	1 000	0 808	0 808	0 192	26
17q21	gain	8q24	gain	5 p/q imbal	gain	LSI 5q31	gain	1 000	0 808	0 808	0 192	26
17q21	gain	8q24	gain	5 p/q imbal	gain	LSI 5p15	gain	1 000	0 808	0 808	0 192	26
17q21	gain	8q24	gain	LSI 5p15	gain	3p14	gain	1 000	0 808	0 808	0 192	26
17q21	gain	8q24	gain	LSI 5p15	gain	CEP 3	gain	1 000	0 808	0 808	0 192	26
17q21	gain	5 p/q imbal	gain	LSI 5q31	gain	3p14	gain	1 000	0 808	0 808	0 192	26
17q21	gain	5 p/q imbal	gain	LSI 5p15	gain	3p14	gain	1 000	0 808	0 808	0 192	26
17q21	gain	5 p/q imbal	gain	LSI 5q31	gain	CEP 3	gain	1 000	0 808	0 808	0 192	26
17q21	gain	5 p/q imbal	gain	LSI 5p15	gain	CEP 3	gain	1 000	0 808	0 808	0 192	26
8q24	gain	CEP 6	gain	5 p/q imbal	gain	LSI 5q31	gain	1 000	0 808	0 808	0 192	26
8q24	gain	CEP 6	gain	5 p/q imbal	gain	LSI 5p15	gain	1 000	0 808	0 808	0 192	26
8q24	gain	CEP 6	gain	LSI 5p15	gain	3p14	gain	1 000	0 808	0 808	0 192	26
8q24	gain	CEP 6	gain	LSI 5p15	gain	CEP 3	gain	1 000	0 808	0 808	0 192	26
8q24	gain	5 p/q imbal	gain	LSI 5q31	gain	3p14	gain	1 000	0 808	0 808	0 192	26
8q24	gain	5 p/q imbal	gain	LSI 5p15	gain	3p14	gain	1 000	0 808	0 808	0 192	26
8q24	gain	5 p/q imbal	gain	LSI 5q31	gain	CEP 3	gain	1 000	0 808	0 808	0 192	26
8q24	gain	5 p/q imbal	gain	LSI 5p15	gain	CEP 3	gain	1 000	0 808	0 808	0 192	26
9p21	gain	8q24	gain	CEP 6	gain	5 p/q imbal	gain	1 000	0 808	0 808	0 192	26
CEP 12	gain	CEP 9	gain	8q24	gain	LSI 5p15	gain	1 000	0 808	0 808	0 192	26
CEP 12	gain	CEP 9	gain	5 p/q imbal	gain	LSI 5p15	gain	1 000	0 808	0 808	0 192	26
CEP 12	gain	CEP 9	gain	8q24	gain	3p14	gain	1 000	0 808	0 808	0 192	26
CEP 12	gain	CEP 9	gain	LSI 5p15	gain	3p14	gain	1 000	0 808	0 808	0 192	26
CEP 12	gain	CEP 9	gain	LSI 5p15	gain	CEP 3	gain	1 000	0 808	0 808	0 192	26
CEP 12	gain	CEP 9	gain	5 p/q imbal	gain	LSI 5q31	gain	1 000	0 808	0 808	0 192	26
CEP 12	gain	CEP 6	gain	5 p/q imbal	gain	LSI 5p15	gain	1 000	0 808	0 808	0 192	26
CEP 12	gain	CEP 6	gain	LSI 5p15	gain	3p14	gain	1 000	0 808	0 808	0 192	26
CEP 12	gain	CEP 6	gain	LSI 5p15	gain	CEP 3	gain	1 000	0 808	0 808	0 192	26
CEP 12	gain	CEP 6	gain	5 p/q imbal	gain	LSI 5q31	gain	1 000	0 808	0 808	0 192	26
CEP 12	gain	8q24	gain	CEP 6	gain	LSI 5p15	gain	1 000	0 808	0 808	0 192	26
CEP 12	gain	8q24	gain	5 p/q imbal	gain	LSI 5p15	gain	1 000	0 808	0 808	0 192	26
CEP 12	gain	8q24	gain	LSI 5p15	gain	3p14	gain	1 000	0 808	0 808	0 192	26
CEP 12	gain	8q24	gain	LSI 5p15	gain	CEP 3	gain	1 000	0 808	0 808	0 192	26
CEP 12	gain	5 p/q imbal	gain	LSI 5q31	gain	3p14	gain	1 000	0 808	0 808	0 192	26
CEP 12	gain	5 p/q imbal	gain	LSI 5p15	gain	3p14	gain	1 000	0 808	0 808	0 192	26
CEP 12	gain	5 p/q imbal	gain	LSI 5q31	gain	CEP 3	gain	1 000	0 808	0 808	0 192	26
CEP 12	gain	5 p/q imbal	gain	LSI 5p15	gain	CEP 3	gain	1 000	0 808	0 808	0 192	26
CEP 6	gain	5 p/q imbal	gain	LSI 5q31	gain	3p14	gain	1 000	0 808	0 808	0 192	26
CEP 6	gain	5 p/q imbal	gain	LSI 5p15	gain	3p14	gain	1 000	0 808	0 808	0 192	26
CEP 6	gain	5 p/q imbal	gain	LSI 5q31	gain	CEP 3	gain	1 000	0 808	0 808	0 192	26
CEP 6	gain	5 p/q imbal	gain	LSI 5p15	gain	CEP 3	gain	1 000	0 808	0 808	0 192	26
CEP 9	gain	8q24	gain	5 p/q imbal	gain	LSI 5q31	gain	1 000	0 808	0 808	0 192	26
CEP 9	gain	8q24	gain	5 p/q imbal	gain	LSI 5p15	gain	1 000	0 808	0 808	0 192	26
CEP 9	gain	8q24	gain	LSI 5p15	gain	3p14	gain	1 000	0 808	0 808	0 192	26
CEP 9	gain	8q24	gain	LSI 5p15	gain	CEP 3	gain	1 000	0 808	0 808	0 192	26
CEP 9	gain	8q24	gain	5 p/q imbal	gain	CEP 1	gain	1 000	0 808	0 808	0 192	26
CEP 9	gain	5 p/q imbal	gain	3p14	gain			1 000	0 808	0 808	0 192	26
CEP 9	gain	5 p/q imbal	gain	LSI 5q31	gain	CEP 3	gain	1 000	0 808	0 808	0 192	26
CEP 9	gain	5 p/q imbal	gain	LSI 5p15	gain	CEP 3	gain	1 000	0 808	0 808	0 192	26

Table 8 Combinations of 2, 3 and 4 Probes at a Cutoff Value of 30%

PROBE 1		PROBE 2		PROBE 3		PROBE 4		SPECIFICITY	SENSITIVITY	SENS*SPEC	VECTOR	# TUMOR SPECIMENS
2 probe combinations												
CEP 6	gain	LSI 5p15	gain					1 000	0 692	0 692	0 308	26
CEP 16	gain	LSI 5p15	gain					1 000	0 684	0 684	0 316	19
LSI 20q	gain	LSI 5p15	gain					1 000	0 684	0 684	0 316	19
LSI 5p15	gain	LSI 3q	gain					1 000	0 684	0 684	0 316	19
17q21	gain	LSI 5p15	gain					1 000	0 654	0 654	0 346	26
7p12	gain	CEP 6	gain					1 000	0 654	0 654	0 346	26
7p12	gain	LSI 5p15	gain					1 000	0 654	0 654	0 346	26
CEP 7	gain	CEP 6	gain					1 000	0 654	0 654	0 346	26
LSI 5p15	gain	3p14	gain					1 000	0 654	0 654	0 346	26
10q23	gain	LSI 5p15	gain					1 000	0 640	0 640	0 360	25
CEP 10	gain	LSI 5p15	gain					1 000	0 640	0 640	0 360	25
LSI 5p15	gain	CEP 4	gain					1 000	0 632	0 632	0 368	19
LSI 5q31	gain	LSI 3q	gain					1 000	0 632	0 632	0 368	19
17p13	gain	LSI 5p15	gain					1 000	0 615	0 615	0 385	26
8q24	gain	LSI 5p15	gain					1 000	0 615	0 615	0 385	26
CEP 17	gain	LSI 5p15	gain					1 000	0 615	0 615	0 385	26
CEP 6	gain	CEP 1	gain					1 000	0 615	0 615	0 385	26
CEP 6	gain	LSI 5q31	gain					1 000	0 615	0 615	0 385	26
CEP 7	gain	LSI 5p15	gain					1 000	0 615	0 615	0 385	26
CEP 8	gain	LSI 5p15	gain					1 000	0 615	0 615	0 385	26
LSI 13	gain	LSI 5p15	gain					1 000	0 615	0 615	0 385	26
LSI 5p15	gain	CEP 1	gain					1 000	0 615	0 615	0 385	26
LSI 5p15	gain	CEP 3	gain					1 000	0 615	0 615	0 385	26
CEP 18	gain	LSI 5p15	gain					1 000	0 600	0 600	0 400	25
7p12	gain	LSI 3q	gain					1 000	0 579	0 579	0 421	19
CEP 16	gain	7p12	gain					1 000	0 579	0 579	0 421	19
CEP 16	gain	LSI 5q31	gain					1 000	0 579	0 579	0 421	19
CEP 7	gain	LSI 3q	gain					1 000	0 579	0 579	0 421	19
LSI 20q	gain	3p14	gain					1 000	0 579	0 579	0 421	19
LSI 20q	gain	7p12	gain					1 000	0 579	0 579	0 421	19
LSI 20q	gain	CEP 12	gain					1 000	0 579	0 579	0 421	19
LSI 20q	gain	CEP 3	gain					1 000	0 579	0 579	0 421	19
LSI 20q	gain	CEP 6	gain					1 000	0 579	0 579	0 421	19
LSI 20q	gain	LSI 5q31	gain					1 000	0 579	0 579	0 421	19
3 probe combinations < 4 and 3 pr comb (1rat + 1 abs)												
8q24	gain	7p12	gain	CEP 6	gain			1 000	0 692	0 692	0 308	26
8q24	gain	CEP 6	gain	LSI 5q31	gain			1 000	0 692	0 692	0 308	26
8q24	gain	CEP 7	gain	CEP 6	gain			1 000	0 692	0 692	0 308	26
CEP 6	gain	5 p/q imbal	gain	LSI 5q31	gain			1 000	0 692	0 692	0 308	26
5 p/q imbal.	gain	LSI 5q31	gain	LSI 3q	gain			1 000	0 684	0 684	0 316	19
8q24	gain	LSI 5q31	gain	LSI 3q	gain			1 000	0 684	0 684	0 316	19
CEP 16	gain	5 p/q imbal	gain	LSI 5q31	gain			1 000	0 684	0 684	0 316	19
LSI 20q	gain	5 p/q imbal	gain	LSI 5q31	gain			1 000	0 684	0 684	0 316	19
17p13	gain	8q24	gain	LSI 5p15	gain			1 000	0 654	0 654	0 346	26
17p13	gain	CEP 17	gain	LSI 5p15	gain			1 000	0 654	0 654	0 346	26
17p13	gain	CEP 7	gain	LSI 5p15	gain			1 000	0 654	0 654	0 346	26
17p13	gain	CEP 8	gain	LSI 5p15	gain			1 000	0 654	0 654	0 346	26
17p13	gain	LSI 13	gain	LSI 5p15	gain			1 000	0 654	0 654	0 346	26
17p13	gain	LSI 5p15	gain	CEP 3	gain			1 000	0 654	0 654	0 346	26
17p13	gain	LSI 5p15	gain	CEP 1	gain			1 000	0 654	0 654	0 346	26
17p13/CEP 17	loss	LSI 5p15	gain					1 000	0 654	0 654	0 346	26
17q21	gain	5 p/q imbal	gain	LSI 5q31	gain			1 000	0 654	0 654	0 346	26
17q21	gain	CEP 6	gain	LSI 5q31	gain			1 000	0 654	0 654	0 346	26
5 p/q imbal.	gain	LSI 5q31	gain	3p14	gain			1 000	0 654	0 654	0 346	26
7p12	gain	5 p/q imbal	gain	LSI 5q31	gain			1 000	0 654	0 654	0 346	26
7p12/CEP 7	gain	CEP 7	gain	LSI 5p15	gain			1 000	0 654	0 654	0 346	26
8q24	gain	CEP 6	gain	CEP 1	gain			1 000	0 654	0 654	0 346	26
9p21	gain	CEP 6	gain	CEP 1	gain			1 000	0 654	0 654	0 346	26

CEP 12	gain	CEP 6	gain	LSI 5q31	gain			1 000	0 654	0 654	0 346	26
CEP 12	gain	CEP 6	gain	LSI 5q31	gain			1 000	0 654	0 654	0 346	26
CEP 17	gain	CEP 6	gain	LSI 5q31	gain			1 000	0 654	0 654	0 346	26
CEP 6	gain	LSI 5q31	gain	CEP 3	gain			1 000	0 654	0 654	0 346	26
CEP 6	gain	LSI 5q31	gain	CEP 1	gain			1 000	0 654	0 654	0 346	26
CEP 8	gain	CEP 6	gain	LSI 5q31	gain			1 000	0 654	0 654	0 346	26
CEP 9	gain	CEP 6	gain	CEP 1	gain			1 000	0 654	0 654	0 346	26
10q23	gain	5 p/q imbal	gain	LSI 5q31	gain			1 000	0.640	0 640	0 360	25
CEP 10	gain	5 p/q imbal	gain	LSI 5q31	gain			1 000	0 640	0 640	0 360	25
CEP 18	gain	17p13	gain	LSI 5p15	gain			1 000	0 640	0 640	0 360	25
4 probe combinations < 4 and 4 pr comb (1 rat + 2 abs)												
17p13/CEP 17	loss	CEP 6	gain	LSI 5p15	gain			1 000	0 731	0 731	0 269	26
17p13/CEP 17	loss	CEP 6	gain	LSI 5q31	gain			1 000	0 692	0 692	0 308	26
17p13/CEP 17	loss	CEP 7	gain	CEP 6	gain			1 000	0 692	0 692	0 308	26
7p12	gain	CEP 6	gain	5 p/q imbal	gain			1 000	0 692	0 692	0 308	26
9p21	gain	8q24	gain	CEP 6	gain	CEP 1	gain	1 000	0 692	0 692	0 308	26
CEP 7	gain	CEP 6	gain	5 p/q imbal	gain			1 000	0 692	0 692	0 308	26
CEP 9	gain	8q24	gain	CEP 6	gain	CEP 1	gain	1 000	0 692	0 692	0 308	26
8q24	gain	7p12	gain	LSI 3q	gain	3p14	gain	1 000	0 684	0 684	0 316	19
8q24	gain	7p12	gain	LSI 3q	gain	CEP 3	gain	1 000	0 684	0 684	0 316	19
8q24	gain	CEP 7	gain	LSI 3q	gain	3p14	gain	1 000	0 684	0 684	0 316	19
8q24	gain	CEP 7	gain	LSI 3q	gain	CEP 3	gain	1 000	0 684	0 684	0 316	19
CEP 12	gain	8q24	gain	7p12	gain	LSI 3q	gain	1 000	0 684	0 684	0 316	19
CEP 12	gain	8q24	gain	CEP 7	gain	LSI 3q	gain	1 000	0 684	0 684	0 316	19
CEP 16	gain	8q24	gain	7p12	gain	LSI 5q31	gain	1 000	0 684	0 684	0 316	19
CEP 16	gain	8q24	gain	7p12	gain	3p14	gain	1 000	0 684	0 684	0 316	19
CEP 16	gain	8q24	gain	CEP 7	gain	3p14	gain	1 000	0 684	0 684	0 316	19
CEP 16	gain	8q24	gain	LSI 5q31	gain	3p14	gain	1 000	0 684	0 684	0 316	19
CEP 16	gain	8q24	gain	7p12	gain	CEP 3	gain	1 000	0 684	0 684	0 316	19
CEP 16	gain	8q24	gain	CEP 7	gain	CEP 3	gain	1 000	0 684	0 684	0 316	19
CEP 16	gain	8q24	gain	LSI 5q31	gain	CEP 3	gain	1 000	0 684	0 684	0 316	19
CEP 16	gain	CEP 11	gain	8q24	gain	LSI 5q31	gain	1 000	0 684	0 684	0 316	19
CEP 16	gain	CEP 12	gain	8q24	gain	7p12	gain	1 000	0 684	0 684	0 316	19
CEP 16	gain	CEP 12	gain	8q24	gain	CEP 7	gain	1 000	0 684	0 684	0 316	19
CEP 16	gain	CEP 12	gain	8q24	gain	LSI 5q31	gain	1 000	0 684	0 684	0 316	19
LSI 20q	gain	8q24	gain	7p12	gain	LSI 5q31	gain	1 000	0 684	0 684	0 316	19
LSI 20q	gain	8q24	gain	7p12	gain	3p14	gain	1 000	0 684	0 684	0 316	19
LSI 20q	gain	8q24	gain	CEP 7	gain	3p14	gain	1 000	0 684	0 684	0 316	19
LSI 20q	gain	8q24	gain	LSI 5q31	gain	3p14	gain	1 000	0 684	0 684	0 316	19
LSI 20q	gain	8q24	gain	7p12	gain	CEP 3	gain	1 000	0 684	0 684	0 316	19
LSI 20q	gain	8q24	gain	CEP 7	gain	CEP 3	gain	1 000	0 684	0 684	0 316	19
LSI 20q	gain	8q24	gain	LSI 5q31	gain	CEP 3	gain	1 000	0 684	0 684	0 316	19
LSI 20q	gain	9p21	gain	8q24	gain	CEP 6	gain	1 000	0 684	0 684	0 316	19
LSI 20q	gain	9p21	gain	8q24	gain	3p14	gain	1 000	0 684	0 684	0 316	19
LSI 20q	gain	9p21	gain	8q24	gain	CEP 3	gain	1 000	0 684	0 684	0 316	19
LSI 20q	gain	CEP 11	gain	8q24	gain	LSI 5q31	gain	1 000	0 684	0 684	0 316	19
LSI 20q	gain	CEP 12	gain	9p21	gain	8q24	gain	1 000	0 684	0 684	0 316	19
LSI 20q	gain	CEP 12	gain	CEP 9	gain	8q24	gain	1 000	0 684	0 684	0 316	19
LSI 20q	gain	CEP 12	gain	8q24	gain	7p12	gain	1 000	0 684	0 684	0 316	19
LSI 20q	gain	CEP 12	gain	8q24	gain	CEP 7	gain	1 000	0 684	0 684	0 316	19
LSI 20q	gain	CEP 12	gain	8q24	gain	LSI 5q31	gain	1 000	0 684	0 684	0 316	19
LSI 20q	gain	CEP 9	gain	8q24	gain	CEP 6	gain	1 000	0 684	0 684	0 316	19
LSI 20q	gain	CEP 9	gain	8q24	gain	3p14	gain	1 000	0 684	0 684	0 316	19
LSI 20q	gain	CEP 9	gain	8q24	gain	CEP 3	gain	1 000	0 684	0 684	0 316	19

Table 9 Combinations of 2, 3 and 4 Probes at a Cutoff Value of 40%

PROBE 1		PROBE 2		PROBE 3		PROBE 4	SPECIFICITY	SENSITIVIT	SENS*SPEC	VECTOR	# TUMOR SPECIMENS
2 probe combinations											
7p12	gain	LSI 3q	gain				1 000	0 579	0 579	0 421	19
7p12	gain	CEP 6	gain				1 000	0 538	0 538	0 462	26
LSI 3q	gain	CEP 1	gain				1 000	0 526	0 526	0 474	19
CEP 6	gain	CEP 1	gain				1 000	0 500	0 500	0 500	26
CEP 7	gain	CEP 6	gain				1 000	0 500	0 500	0 500	26
CEP 18	gain	7p12	gain				1 000	0 480	0 480	0 520	25
7p12	gain	CEP 4	gain				1 000	0 474	0 474	0 526	19
CEP 16	gain	7p12	gain				1 000	0 474	0 474	0 526	19
CEP 7	gain	LSI 3q	gain				1 000	0 474	0 474	0 526	19
LSI 20q	gain	7p12	gain				1 000	0 474	0 474	0 526	19
LSI 5p15	gain	LSI 3q	gain				1 000	0 474	0 474	0 526	19
7p12	gain	LSI 5p15	gain				1 000	0 462	0 462	0 538	26
CEP 10	gain	7p12	gain				1 000	0 440	0 440	0 560	25
CEP 18	gain	CEP 1	gain				1 000	0 440	0 440	0 560	25
7p12	gain	LSI 5q31	gain				1 000	0 423	0 423	0 577	26
CEP 11	gain	7p12	gain				1 000	0 423	0 423	0 577	26
CEP 6	gain	LSI 5p15	gain				1 000	0 423	0 423	0 577	26
CEP 7	gain	LSI 5p15	gain				1 000	0 423	0 423	0 577	26
LSI 5p15	gain	CEP 1	gain				1 000	0 423	0 423	0 577	26
CEP 16	gain	CEP 1	gain				1 000	0 421	0 421	0 579	19
CEP 16	gain	CEP 7	gain				1 000	0 421	0 421	0 579	19
CEP 4	gain	CEP 1	gain				1 000	0 421	0 421	0 579	19
LSI 20q	gain	CEP 1	gain				1 000	0 421	0 421	0 579	19
LSI 20q	gain	CEP 7	gain				1 000	0 421	0 421	0 579	19
10q23	gain	7p12	gain				1 000	0 400	0 400	0 600	25
CEP 10	gain	CEP 1	gain				1 000	0 400	0 400	0 600	25
CEP 18	gain	CEP 7	gain				1 000	0 400	0 400	0 600	25
CEP 11	gain	CEP 1	gain				1 000	0 385	0 385	0 615	26
CEP 11	gain	CEP 7	gain				1 000	0 385	0 385	0 615	26
CEP 12	gain	CEP 6	gain				1 000	0 385	0 385	0 615	26
3 probe combinations											
CEP 11	gain	7p12	gain	CEP 6	gain		1 000	0 577	0 577	0 423	26
CEP 11	gain	CEP 6	gain	CEP 1	gain		1 000	0 538	0 538	0 462	26
CEP 11	gain	CEP 7	gain	CEP 6	gain		1 000	0 538	0 538	0 462	26
17q21	gain	CEP 7	gain	LSI 3q	gain		1 000	0 526	0 526	0 474	19
CEP 11	gain	7p12	gain	CEP 4	gain		1 000	0 526	0 526	0 474	19
CEP 11	gain	CEP 7	gain	LSI 3q	gain		1 000	0 526	0 526	0 474	19
CEP 16	gain	CEP 11	gain	7p12	gain		1 000	0 526	0 526	0 474	19
CEP 16	gain	CEP 7	gain	LSI 3q	gain		1 000	0 526	0 526	0 474	19
CEP 7	gain	CEP 6	gain	LSI 3q	gain		1 000	0 526	0 526	0 474	19
CEP 7	gain	LSI 5p15	gain	LSI 3q	gain		1 000	0 526	0 526	0 474	19
LSI 20q	gain	CEP 11	gain	7p12	gain		1 000	0 526	0 526	0 474	19
LSI 20q	gain	CEP 7	gain	LSI 3q	gain		1 000	0 526	0 526	0 474	19
CEP 18	gain	10q23	gain	7p12	gain		1 000	0 520	0 520	0 480	25
CEP 18	gain	CEP 10	gain	7p12	gain		1 000	0 520	0 520	0 480	25
CEP 18	gain	CEP 6	gain	CEP 1	gain		1 000	0 520	0 520	0 480	25
CEP 18	gain	CEP 7	gain	CEP 6	gain		1 000	0 520	0 520	0 480	25
CEP 11	gain	7p12	gain	LSI 5p15	gain		1 000	0 500	0 500	0 500	26
CEP 18	gain	7p12	gain	CEP 4	gain		1 000	0 500	0 500	0 500	18
CEP 18	gain	CEP 16	gain	7p12	gain		1 000	0 500	0 500	0 500	18
LSI 20q	gain	CEP 18	gain	7p12	gain		1 000	0 500	0 500	0 500	18
10q23	gain	7p12	gain	LSI 5p15	gain		1 000	0 480	0 480	0 520	25
CEP 10	gain	7p12	gain	LSI 5p15	gain		1 000	0 480	0 480	0 520	25
CEP 11	gain	CEP 10	gain	7p12	gain		1 000	0 480	0 480	0 520	25
CEP 18	gain	10q23	gain	CEP 1	gain		1 000	0 480	0 480	0 520	25
CEP 18	gain	CEP 10	gain	CEP 1	gain		1 000	0 480	0 480	0 520	25
CEP 11	gain	CEP 4	gain	CEP 1	gain		1 000	0 474	0 474	0 526	19
CEP 11	gain	CEP 7	gain	CEP 4	gain		1 000	0 474	0 474	0 526	19
CEP 16	gain	CEP 11	gain	CEP 7	gain		1 000	0 474	0 474	0 526	19
CEP 16	gain	CEP 11	gain	CEP 1	gain		1 000	0 474	0 474	0 526	19
LSI 20q	gain	CEP 11	gain	CEP 7	gain		1 000	0 474	0 474	0 526	19
LSI 20q	gain	CEP 11	gain	CEP 1	gain		1 000	0 474	0 474	0 526	19

4 probe combinations												
17p13/CEP 17	loss	CEP 6	gain	CEP 1	gain			1 000	0 538	0 538	0 462	26
17p13/CEP 17	loss	CEP 7	gain	CEP 6	gain			1 000	0 538	0 538	0 462	26
9p21/CEP 9	loss	CEP 7	gain	LSI 3q	gain			1 000	0 526	0 526	0 474	19
CEP 11	gain	10q23	gain	7p12	gain	LSI 5p15	gain	1 000	0 520	0 520	0 480	25
CEP 11	gain	CEP 10	gain	7p12	gain	LSI 5q31	gain	1 000	0 520	0 520	0 480	25
CEP 11	gain	7p12	gain	5 p/q imbal	gain			1 000	0 500	0 500	0 500	26
CEP 11	gain	CEP 9	gain	CEP 6	gain	LSI 5p15	gain	1 000	0 500	0 500	0 500	26
10q23	gain	7p12	gain	5 p/q imbal	gain			1 000	0 480	0 480	0 520	25
CEP 10	gain	7p12	gain	5 p/q imbal	gain			1 000	0 480	0 480	0 520	25
CEP 11	gain	10q23	gain	7p12	gain	LSI 5q31	gain	1 000	0 480	0 480	0 520	25
CEP 11	gain	10q23	gain	CEP 7	gain	LSI 5p15	gain	1 000	0 480	0 480	0 520	25
CEP 11	gain	10q23	gain	LSI 5p15	gain	CEP 1	gain	1 000	0 480	0 480	0 520	25
CEP 11	gain	CEP 10	gain	CEP 7	gain	LSI 5q31	gain	1 000	0 480	0 480	0 520	25
CEP 11	gain	CEP 10	gain	LSI 5q31	gain	CEP 1	gain	1 000	0 480	0 480	0 520	25
CEP 11	gain	CEP 10	gain	LSI 5p15	gain	CEP 1	gain	1 000	0 480	0 480	0 520	25
CEP 11	gain	CEP 10	gain	CEP 7	gain	LSI 5q31	gain	1 000	0 480	0 480	0 520	25
CEP 11	gain	CEP 10	gain	CEP 7	gain	LSI 5p15	gain	1 000	0 480	0 480	0 520	25
CEP 18	gain	10q23	gain	CEP 7	gain	LSI 5p15	gain	1 000	0 480	0 480	0 520	25
CEP 18	gain	17p13	gain	10q23	gain	CEP 7	gain	1 000	0 480	0 480	0 520	25
CEP 18	gain	17p13	gain	CEP 10	gain	CEP 7	gain	1 000	0 480	0 480	0 520	25
CEP 18	gain	17p13/CEP 17	loss	CEP 1	gain			1 000	0 480	0 480	0 520	25
CEP 18	gain	17q21	gain	10q23	gain	CEP 7	gain	1 000	0 480	0 480	0 520	25
CEP 18	gain	17q21	gain	CEP 10	gain	CEP 7	gain	1 000	0 480	0 480	0 520	25
CEP 18	gain	CEP 10	gain	CEP 7	gain	LSI 5p15	gain	1 000	0 480	0 480	0 520	25
CEP 18	gain	CEP 11	gain	10q23	gain	CEP 7	gain	1 000	0 480	0 480	0 520	25
CEP 18	gain	CEP 11	gain	CEP 10	gain	CEP 7	gain	1 000	0 480	0 480	0 520	25
CEP 18	gain	CEP 9	gain	CEP 6	gain	LSI 5p15	gain	1 000	0 480	0 480	0 520	25
17p13/CEP 17	loss	CEP 6	gain	LSI 5p15	gain			1 000	0 462	0 462	0 538	26
17p13/CEP 17	loss	CEP 7	gain	LSI 5p15	gain			1 000	0 462	0 462	0 538	26
17p13/CEP 17	loss	LSI 5p15	gain	CEP 1	gain			1 000	0 462	0 462	0 538	26
9p21/CEP 9	loss	CEP 6	gain	LSI 5p15	gain			1 000	0 462	0 462	0 538	26
CEP 11	gain	5 p/q imbal	gain	CEP 1	gain			1 000	0 462	0 462	0 538	26
CEP 11	gain	CEP 7	gain	5 p/q imbal	gain			1 000	0 462	0 462	0 538	26
CEP 12	gain	CEP 11	gain	CEP 9	gain	CEP 6	gain	1 000	0 462	0 462	0 538	26

Table 10 Analysis of Bronchial Secretions from 21 Patients by Cytology, Bronchus Biopsy, and FISH

					FISH Result	
Specimen I.D.	**Clinical Diagnosis**	**Cytology Result**	**Bronchus Biopsy**	**Additional Biopsy**	**Probes Indicating Gain**	**FISH Diagnosis**
#3935	Small Cell CA	positive	positive	not done	LSI 5p15	positive
#3912	Squamous Cell CA	positive	positive	not done	LSI 8q24, LSI 5p15, CEP 1 CEP 6	positive
#3911	Squamous Cell CA	positive	positive	not done	LSI 8q24, LSI 5p15, CEP 1 CEP 6	positive
#2870	Mesenchymal CA	negative	negative	positive	LSI 5p15, CEP 6	positive
#30582	Adenocarcinoma	positive	not done	not done	LSI 8q24, LSI 5p15, CEP 1 CEP 6	positive
#1995	Breast CA metastasis	positive	positive	positive	LSI 8q24, LSI 5p15, CEP 1 CEP 6	positive
#2786	Large Cell CA	negative	negative	positive	none	negative
#2789	No malignancy	negative	negative	not done	none	negative
#2545	Small Cell CA	positive	positive	not done	LSI 8q24, LSI 5p15, CEP 1	positive
#3700	Adenocarcinoma	positive	positive	not done	LSI 8q24, LSI 5p15	positive
#2363	Large Cell CA	positive	positive	not done	LSI 8q24, LSI 5p15, CEP 1	positive
#3739	Squamous Cell CA	positive	positive	not done	LSI 8q24, LSI 5p15, CEP 1	positive
#30796	Small Cell CA	positive	positive	not done	LSI 8q24, LSI 5p15	positive
#30671	Adenocarcinoma	positive	negative	positive	LSI 8q24, LSI 5p15	positive
#1864	Breast CA metastasis	positive	negative	positive	LSI 8q24, LSI 5p15	positive
#2546	Large Cell CA	negative	not done	positive	-	not evaluated*
#2577	No malignancy	negative	negative	not done	none	negative
#2251	No malignancy	negative	not done	negative	none	negative
#2603	No malignancy	negative	negative	not done	none	negative
#2785	No malignancy	negative	negative	pos for epipharynx CA	none	negative
#30706	Equivocal	negative	Not done	Equivocal	none	negative

*cell morphology was too poor to permit evaluation.

Table 11 Conventional Cytology Performance Compared to Clinical Diagnosis

Cytology Result	Clinical Diagnosis	
	negative	positive/equivocal
negative	5	4
positive	0	12

specificity = 100%
sensitivity = 75%
sensitivity = 80% excluding the slide not evaluated by FISH

Table 12 FISH Performance Compared to Clinical Diagnosis

FISH Result	Clinical Diagnosis	
	negative	positive/equivocal
negative	5	2
positive	0	13
not evaluated	0	1

specificity = 100%
sensitivity = 81% including non-evaluable FISH slide
sensitivity = 87% excluding non-evaluable FISH slide

Table 13 Probe Sets Based on Discriminate and Combinatorial Analyses

				VECTOR VALUE				
PROBE 1	PROBE 2	PROBE 3	PROBE 4	CUTOFF = 5	CUTOFF = 10	CUTOFF = 20	CUTOFF = 3	CUTOFF = 40
Single probes:								
LSI 5p15				0.407	0 231	0.346	0 423	0.692
CEP 1				0.077	0.346	0.462	0.615	0 654
CEP 6				0.287	0.385	0 500	0.500	0.692
LSI 7p12				0.619	0.324	0.385	0 500	0.615
LSI 8q24				0.210	0 222	0.556	0.778	0 889
CEP 9				0.287	0.346	0 577	0.808	0 885
2 Probe combinations:								
LSI 5p15	LSI 8q24				0.154	0.269	0 385	
LSI 5p15	LSI 3q					0.211	0 316	0.526
LSI 5p15	LSI 20q					0 263	0.316	
LSI 5p15	LSI 7p12					0.308	0.346	0.538
LSI 5p15	CEP 16					0 263	0.316	
LSI 5p15	CEP 4					0 263	0 368	
LSI 5p15	CEP 12				0.154	0.308	0.368	
LSI 5p15	CEP 6					0 269	0.308	0 577
LSI 5p15	LSI 17q21				0.192	0.269	0.346	
LSI 8q24	CEP 17				0.148			
LSI 8q24	CEP 1				0.154			
LSI 8q24	CEP 6				0.192	0.308		
LSI 7p12	LSI 3q					0.316	0 421	0.421
LSI 7p12	CEP 6						0.346	0 462
LSI 3q	CEP 7					0.316	0.421	0.526
CEP 6	CEP 7						0 346	0 500
3 Probe combinations:								
LSI 5p15	LSI 8q24	LSI 9p21			0 115			
LSI 5p15	CEP 12	LSI 9p21			0.115			
LSI 8q24	CEP 17	LSI 9p21			0 115			
LSI 8q24	CEP 1	LSI 9p21			0 115			
LSI 5p15	LSI 3q	CEP 12				0.158		
4 Probe combinations:								
LSI 5p15	CEP 6	LSI 17p13 (loss)	CEP 17				0 269	
Probe sets with redundant complementation:								
3 probe combinations (sum of 2 probe pairs with 1 probe in common):								
LSI 5p15	LSI 8q24	LSI 3q						
LSI 5p15	LSI 8q24	LSI 20q						
LSI 5p15	LSI 8q24	LSI 7p12						
LSI 5p15	LSI 8q24	CEP 16						
LSI 5p15	LSI 8q24	CEP 4						

LSI 5p15	LSI 8q24	CEP 12								
LSI 5p15	LSI 8q24	CEP 6								
LSI 5p15	LSI 8q24	LSI 17q21								
LSI 5p15	LSI 8q24	CEP 17								
LSI 5p15	LSI 8q24	CEP 1								
LSI 5p15	LSI 3q	LSI 20q								
LSI 5p15	LSI 3q	LSI 7p12								
LSI 5p15	LSI 3q	CEP 16								
LSI 5p15	LSI 3q	CEP 4								
LSI 5p15	LSI 3q	CEP 12								
LSI 5p15	LSI 3q	CEP 6								
LSI 5p15	LSI 3q	LSI 17q21								
LSI 5p15	LSI 3q	CEP 7								
LSI 5p15	LSI 3q	LSI 7p12								
LSI 5p15	LSI 20q	LSI 7p12								
LSI 5p15	LSI 20q	CEP 16								
LSI 5p15	LSI 20q	CEP 4								
LSI 5p15	LSI 20q	CEP 12								
LSI 5p15	LSI 20q	CEP 6								
LSI 5p15	LSI 20q	LSI 17q21								
LSI 5p15	LSI 7p12	CEP 16								
LSI 5p15	LSI 7p12	CEP 4								
LSI 5p15	LSI 7p12	CEP 12								
LSI 5p15	LSI 7p12	CEP 6								
LSI 5p15	LSI 7p12	LSI 17q21								
LSI 5p15	LSI 7p12	LSI 3q								
LSI 5p15	LSI 7p12	CEP 6								
LSI 5p15	CEP 16	CEP 4								
LSI 5p15	CEP 16	CEP 12								
LSI 5p15	CEP 16	CEP 6								
LSI 5p15	CEP 16	LSI 17q21								
LSI 5p15	CEP 4	CEP 12								
LSI 5p15	CEP 4	CEP 6								
LSI 5p15	CEP 4	LSI 17q21								
LSI 5p15	CEP 12	CEP 6								
LSI 5p15	CEP 12	LSI 17q21								
LSI 5p15	CEP 6	LSI 17q21								
LSI 5p15	CEP 6	CEP 7								
LSI 8q24	CEP 17	CEP 1								
LSI 8q24	CEP 17	CEP 6								
LSI 8q24	CEP 1	CEP 6								
LSI 8q24	LSI 7p12	CEP 6								
LSI 8q24	CEP 6	CEP 7								
LSI 7p12	LSI 3q	CEP 6								
LSI 7p12	LSI 3q	CEP 7								
LSI 7p12	CEP 6	CEP 7								
LSI 3q	CEP 6	CEP 7								

4 probe combinations - 2 redundant complementary pairs:

LSI 5p15	LSI 8q24	7p12	LSI 3q							
LSI 5p15	LSI 8q24	7p12	CEP 6							
LSI 5p15	LSI 8q24	LSI 3q	CEP 7							
LSI 5p15	LSI 8q24	CEP 6	CEP 7							
LSI 5p15	LSI 3q	8q24	CEP 17							

LSI 5p15	LSI 3q	8q24	CEP 1
LSI 5p15	LSI 3q	8q24	CEP 6
LSI 5p15	LSI 3q	7p12	CEP 6
LSI 5p15	LSI 3q	CEP 6	CEP 7
LSI 5p15	LSI 20q	8q24	CEP 17
LSI 5p15	LSI 20q	8q24	CEP 1
LSI 5p15	LSI 20q	8q24	CEP 6
LSI 5p15	LSI 20q	7p12	LSI 3q
LSI 5p15	LSI 20q	7p12	CEP 6
LSI 5p15	LSI 20q	LSI 3q	CEP 7
LSI 5p15	LSI 20q	CEP 6	CEP 7
LSI 5p15	7p12	8q24	CEP 17
LSI 5p15	7p12	8q24	CEP 1
LSI 5p15	7p12	8q24	CEP 6
LSI 5p15	7p12	LSI 3q	CEP 7
LSI 5p15	7p12	CEP 6	CEP 7
LSI 5p15	CEP 16	LSI 8q24	CEP 17
LSI 5p15	CEP 16	LSI 8q24	CEP 1
LSI 5p15	CEP 16	LSI 8q24	CEP 6
LSI 5p15	CEP 16	LSI 7p12	LSI 3q
LSI 5p15	CEP 16	LSI 7p12	CEP 6
LSI 5p15	CEP 16	LSI 3q	CEP 7
LSI 5p15	CEP 16	CEP 6	CEP 7
LSI 5p15	CEP 4	LSI 8q24	CEP 17
LSI 5p15	CEP 4	LSI 8q24	CEP 1
LSI 5p15	CEP 4	LSI 8q24	CEP 6
LSI 5p15	CEP 4	LSI 7p12	LSI 3q
LSI 5p15	CEP 4	LSI 7p12	CEP 6
LSI 5p15	CEP 4	LSI 3q	CEP 7
LSI 5p15	CEP 4	CEP 6	CEP 7
LSI 5p15	CEP 12	LSI 8q24	CEP 17
LSI 5p15	CEP 12	LSI 8q24	CEP 1
LSI 5p15	CEP 12	LSI 8q24	CEP 6
LSI 5p15	CEP 12	LSI 7p12	LSI 3q
LSI 5p15	CEP 12	LSI 7p12	CEP 6
LSI 5p15	CEP 12	LSI 3q	CEP 7
LSI 5p15	CEP 12	CEP 6	CEP 7
LSI 5p15	CEP 6	LSI 8q24	CEP 17
LSI 5p15	CEP 6	LSI 8q24	CEP 1
LSI 5p15	CEP 6	LSI 7p12	LSI 3q
LSI 5p15	CEP 6	LSI 3q	CEP 7
LSI 5p15	LSI 17q21	LSI 8q24	CEP 17
LSI 5p15	LSI 17q21	LSI 8q24	CEP 1
LSI 5p15	LSI 17q21	LSI 8q24	CEP 6
LSI 5p15	LSI 17q21	LSI 7p12	LSI 3q
LSI 5p15	LSI 17q21	LSI 7p12	CEP 6
LSI 5p15	LSI 17q21	LSI 3q	CEP 7
LSI 5p15	LSI 17q21	CEP 6	CEP 7
LSI 8q24	CEP 17	LSI 7p12	LSI 3q
LSI 8q24	CEP 17	LSI 7p12	CEP 6
LSI 8q24	CEP 17	LSI 3q	CEP 7
LSI 8q24	CEP 17	CEP 6	CEP 7
LSI 8q24	CEP 1	LSI 7p12	LSI 3q
LSI 8q24	CEP 1	LSI 7p12	CEP 6
LSI 8q24	CEP 1	LSI 3q	CEP 7

LSI 8q24	CEP 1	CEP 6	CEP 7					
LSI 8q24	CEP 6	LSI 7p12	LSI 3q					
LSI 8q24	CEP 6	LSI 3q	CEP 7					
LSI 7p12	LSI 3q	CEP 6	CEP 7					
LSI 7p12	CEP 6	LSI 3q	CEP 7					
4 probe combinations - 3 pairs with 2 common probes.								
examples:								
LSI 5p15	LSI 8q24	LSI 3q	CEP 1	(probe pairs in rows 17 +18+27)				
LSI 5p15	LSI 8q24	LSI 3q	CEP 6	(probe pairs in rows 17 +18+28)				
LSI 5p15	LSI 8q24	CEP 1	CEP 6	(probe pairs in rows 17 +24+27)				
LSI 7p12	LSI 3q	CEP 6	CEP 7	(probe pairs in rows 29 +30+31)				

plus 3 probe labels) looking for cells with target gains. The number of targets for each of the 3 probes was recorded for any cell showing gain in one or more of the 3 targets.

Example 5: Detection of Lung Cancer in Bronchial Washing Specimens

The present study used an interphase FISH assay (using a 4-probe multicolor FISH panel) to detect lung cancer in 74 bronchial washing specimens that had previously been characterized by cytological analysis. Forty eight of the specimens were from patients with a clinical diagnosis of positive for cancer, and 26 of the specimens were from patients with a clinical diagnosis of negative for cancer.

Bronchial washing specimens were selected from the cytopathology archives of the Institute of Pathology in Basel, Switzerland. These cytology specimens were pre-stained with PAP stain and permanently mounted under coverslips. Specimens were archived for a period of time ranging from a few months to two years.

The four probes used for the FISH assay included a repetitive sequence probe centromeric to chromosome 1 (CEP 1), and three unique-sequence probes to the loci 5p15, 8q24 (containing the c-myc gene), and 7p12 (containing the EGFR gene), labeled respectively with SpectrumAqua™, SpectrumGreen™, SpectrumGold™, and SpectrumRed™. The probes were mixed together and hybridized simultaneously to each bronchial wash specimen.

The archived slides were soaked in xylene until the coverslips fell off (approximately 4-5 days) and then washed in fresh xylene twice, 5 minutes per wash. The slides were then placed in 95% ethanol, 85% ethanol, and 70% ethanol, sequentially (5 minutes per solution), followed by soaking the slides in 2xSSC buffer for 1 minute. The slides were then incubated in 0.5 mg/ml pepsin solution in 10 mM HCl for 10 minutes at 37°C, followed by a PBS wash for 5 minutes. The slides were fixed in a freshly prepared solution of 1% neutral buffered formalin for 5 minutes at 4°C, followed by soaking in PBS for 5 minutes. The slides were then denatured for 10 minutes in 70% formamide/2xSSC at 73°C, dehydrated in an ethanol series of 70%, 85%, and 100% ethanol (5 minutes per solution), and put on a slide warmer at 37-45°C for 1 minute to dry. Probes in the hybridization mixture were denatured by placing the tube containing the mixture in a 73°C water bath for 5 minutes. The denatured probe hybridization

mixtures were applied to the specimens, covered with coverslips, and sealed with rubber cement. The slides were incubated at 37°C overnight, after which the slides were washed in 2xSSC/0.3% NP40 at 73°C for 2-5 minutes. The slides were then placed in 2xSSC/0.1% NP40 for several seconds to several minutes. DAPI II was applied to the target areas and the slides were analyzed under the fluorescence microscope using single bandpass filter sets.

The specimen slides were evaluated under a fluorescence microscope to first assess the technical quality of the FISH signals and the background staining. If the quality was acceptable, the slides were then enumerated. The overall sample appearance was evaluated with a DAPI single bandpass filter set at 40x magnification. The following sample features were important to note: 1) the presence of thin or thick mucous fibers; 2) the degree to which the cells were trapped within mucous fibers; 3) the presence of nuclear pleomorphism; and 4) the presence of disrupted cells (no clear nuclear borders, amorphous shape). Cells or groups of cells were selected for signal enumeration only if they had clearly defined nuclear borders and preferably were in the areas free of mucous fibers.

Enumeration was carried out according to the following rules using the DAPI single bandpass filter set and the three probe-specific single bandpass filter sets (Vysis aqua, green, gold, and red). All specimen evaluations were performed with the reviewer blinded to the identity of the specimen.

(1) Select the appropriate area with cells using the DAPI single bandpass filter set.

(2) Change to the gold or green single bandpass filter set and observe the field. If cells with signal copy gain are present, record the copy number pattern in those cells for all 4 probes, changing sequentially to the other three probe-specific single bandpass filter sets (order not important). If the cells look disomic with the gold or green filter set, change to one of the other three probe-specific filter sets and observe the field. If cells with signal copy gains are present, record their signal pattern for all 4 probes. Do this until the field has been scanned with all 4 probe-specific filter sets. Only record the pattern for any one cell once.

(3) Move to a new area and repeat the evaluation.

(4) Stop enumeration when at least 25 cells are scored or the end of the slide was reached.

Enumeration results of signal copy number for each probe were analyzed using JMP 3.2 version statistical software.

The samples used in this study were selected so that approximately half of the 48 specimens with a clinical diagnosis of cancer were also diagnosed as positive by cytology, and approximately half were diagnosed as negative by cytology. The majority of the cancer positive specimens were from patients with adenocarcinoma (23 specimens), followed by patients with squamous cell carcinoma (11 specimens). The rest of the specimens were from patients with large cell carcinoma (6 specimens), small cell carcinoma (6 specimens), carcinoid tumor (1 specimen), and leiomyosarcoma (1 specimen). All 26 specimens clinically negative for cancer had negative cytology results. No specimens were selected with a negative clinical diagnosis and a positive cytology result (the cytology specificity in this study was 100% by design).

Table 14 shows the distribution of the cytology results in the cohort of patients that was used in this study. The cytology results were positive for 22 patients, negative for 48 patients and suspicious for 4 patients. The sensitivity of cytology for the group of 48 samples positive for cancer by clinical diagnosis was 45.8%. Thirteen specimens were rejected from FISH evaluation due to the excessive loss of tissue (9 specimens from cancer positive patients and 4 specimens from cancer negative patients). Excluding the slides that were not evaluated by FISH, the cytology sensitivity for the remaining 39 cancer positive patients was 50%. If cytology suspicious samples were counted as positive, the cytology sensitivity increased to 53.9%.

Table 14 Correlation Between Cytology Results and Clinical Diagnosis

	Clinical Diagnosis	
Cytology	Cancer Negative	Cancer Positive
Cytology Negative	26 (100%)	22 (45.83%)
Cytology Positive	0 (0%)	22 (45.83%)
Cytology Suspicious	0 (0%)	4 (8.33%)

The bronchial washing specimens were hybridized with the multicolor FISH probe mixture after the coverslips were removed by soaking in xylene. The overall appearance of each sample was evaluated. If the specimen appeared to be extremely acellular or the morphology of the cells was disturbed, or the hybridization signal was too weak, then the sample was rejected for FISH enumeration.

To evaluate the FISH results, it was necessary to develop a cancer positivity criteria. This involved developing rules to classify individual cells as being suspicious for malignancy ("abnormal") or not suspicious ("normal"), and setting cutoff values for the minimum number of abnormal cells required to classify a specimen as positive for cancer.

A cell was classified as abnormal if it showed copy number gains for at least two probes included in the probe mix (this was termed "Multiple DNA loci gain"). Once this rule was established, all of the specimen data were evaluated and the number of "abnormal" cells in each of the specimens was tabulated. To decide what should be the "cancer positivity criteria" (a quantitative measure to discern cancer negative from cancer positive cases), the receiver operator characteristic (ROC) curve approach was applied to the data analysis. Using this approach, a series of tentative cutoff points are set and the sensitivity and specificity are calculated at each point. For data presented here, cutoff values of 1 to 10 cells per specimen were used. For each cutoff value the sensitivity was determined for the cohort of cancer positive patients, and the specificity was determined for the cohort of cancer negative patients. Then the ROC curve was plotted for sensitivity (y axis) as a function of [1- specificity] (x axis) (Figure 1).

As seen in Figure 1, there is a section on the curve, where the sensitivity increases significantly while specificity remains about the same. The cutoff point is often selected in the section where the curve turns. The turning point in this assay corresponded to a cutoff value of finding 5-6 cells that met the criteria of cancer positivity. Consequently, the rule for classifying a specimen as positive used in this study was as follows: if a sample contained 6 or more abnormal cells with "multiple loci gain," it was classified as "cancer positive." If a sample had less than 6 abnormal cells, it was classified as "cancer negative."

Table 15 shows the correlation between cytology and FISH results for the group of "cancer positive" patients. Cytology was positive in 22 out of 48 "cancer positive" patients, providing a sensitivity of 45.8%. For another 4 specimens the cytology was reevaluated by cytopathologists, and the specimens classified as "suspicious". If "suspicious" results were interpreted as "cancer positive", then the sensitivity of cytology became 53.8%. Several samples were rejected from FISH evaluation due to low cellularity and other reasons, so the number of cases evaluated by FISH was different from the number of cases evaluated by cytology. Recalculating the cytology results for those cases that were also evaluated by FISH, the sensitivity of cytology became 46.2% (18/39 cases), if "suspicious" results are counted as positive results, the sensitivity would be 53.8%. Thus, there was no significant difference between the sensitivity results if FISH-rejected samples were included or excluded from the calculations. The FISH results for the same group of patients showed 32 positive results among the 39 "cancer positive" patients, providing a sensitivity of 82.0%.

Table 15 Cancer Positive Patients: Correlation of FISH and Cytology Results

	FISH Negative	FISH Positive	FISH Rejected	Total
Cytology Negative	3	15	4	22
Cytology Positive	3	15	4	22
Cytology Suspicious	1	2	1	4
Total	7	32	9	48

FISH was able to clarify two of the cytology suspicious specimens (an additional specimen was rejected for FISH evaluation) by placing them into the category of "cancer positive" specimens. The number of abnormal cells in each of those specimens was 8 for a small cell carcinoma specimen and 10 for a large cell carcinoma specimen. Even more important are the results obtained for the group of 18 cytology negative/cancer positive cases. Table 15 shows that for these cancer patients that were missed by cytology, FISH was positive in 15/18 cases, thus improving the diagnosis in 83.3% of cases.

FISH and cytology results were also analyzed relative to the type of tumor. The data showed that FISH had its lowest sensitivity for the specimens diagnosed as squamous cell carcinoma (5/9 specimens, 55.5%). For this type of lung tumor, cytology showed 54.5% sensitivity. Adenocarcinoma, large cell carcinoma, and small cell carcinoma demonstrated sensitivity by FISH of 86.4% (19/22 cases), 100% (5/5 cases) and 100% (3/3 cases), respectively. Cytology sensitivity for these tumors was as follows: 60.9% for adenocarcinoma; 50% for large cell carcinoma; and 100% for small cell carcinoma.

The group of "cancer negative" patients consisted of 26 patients. Cytology results were negative for all of the patients in this selected group setting the specificity of 100%. Four specimens were rejected from FISH evaluation due to low cellularity, thus only 22 specimens were evaluated. Among those 22 specimens, FISH was clearly negative in 18 patients providing a specificity of 81.8% (Table 16). Four specimens had positive FISH results. These four specimens contained as many as 19, 15, 11 and 8 "abnormal" cells per 25 evaluated suspicious cells. It is also important to note that in two of the specimens, the magnitude of copy number gain was as high as 7-8 copies per cell in one case and 11-12 copies per cell in another case. One of the specimens was derived from a patient diagnosed with advanced colorectal cancer approximately one year before the specimen was prepared (the patient died by the time of the present study). Another patient had a previous history of heavy smoking and had the occupational hazard of being a miner. Thus, it is possible that these FISH positive, but "cytology negative" specimens were derived from patients at risk of developing lung cancer.

Table 16 Cancer Negative Patients: Correlation of FISH and Cytology Results

	FISH Negative	FISH Positive	FISH Rejected	Total
Cytology Negative	18	4	4	26
Cytology Positive/Suspicious	0	0	0	0
Total	18	4	4	26

Table 17 shows comparative data on sensitivity and specificity for cytology and FISH for the total population of 74 patients.

Table 17 Total population of patients: Correlation of FISH and cytology results

	FISH Negative	FISH Positive	FISH Rejected	Total
Cytology Negative	21	19	8	48
Cytology Positive	3	15	4	22
Cytology Suspicious	1	2	1	4
Total	25	36	13	74

Example 6: Detection of Lung Cancer in Bronchoscopic Specimens

The present study used an interphase FISH assay (using a 4-probe multicolor FISH panel) to detect lung cancer in 191 bronchial specimens that had previously been characterized by surgical pathology analysis. The surgical pathology results of the specimens used in this study are summarized in Table 18. 104 of the specimens (55%) were from patients with a clinical diagnosis of positive for lung cancer. 84 of the specimens (44%) were from patients with a clinical diagnosis of negative for lung cancer.

Table 18 Surgical Pathology Results of Specimens Used in Study

Number of Specimens	Diagnosis (+ or – for cancer)	Percentage
104	+	55
84	-	44
3	Equivocal diagnosis	1

One of the following three sets of four probes was used for each FISH assay: (1) a repetitive sequence probe centromeric to chromosome 1 (CEP 1), and three unique-sequence probes to the loci 5p15, 8q24, and 7p12; (2) repetitive sequence probes centromeric to chromosome 16 (CEP 16) and chromosome 17 (CEP 17) and two unique-

sequence probes to the loci 3q26 and 20q13; or (3) a repetitive sequence probe centromeric to chromosome 6 (CEP 6) and three unique-sequence probes to the loci 5p15, 8q24, and 7p12. The probes were mixed together and hybridized simultaneously to each bronchial specimen.

The sensitivity detected by each of FISH and cytology analysis for the 104 cancer positive specimens is depicted in Table 19 (38 bronchial brushing samples) and Table 20 (66 bronchial secretion samples). As shown in Table 19, FISH demonstrated a significantly enhanced sensitivity (72%) as compared to cytology (51%) for the bronchial brushing samples. No significant difference between FISH and cytology was detected for the bronchial secretion samples (Table 20).

Table 19 Sensitivity of FISH and Cytology for Bronchial Brushing Samples

Analysis	Diagnosis	Number	Percentage
FISH	+	26/36	72
FISH	-	8/36	22
FISH	Equivocal diagnosis	2/36	6
Cytology	+	19/37	51
Cytology	-	17/37	46
Cytology	Equivocal diagnosis	1/37	3

Table 20 Sensitivity of FISH and Cytology for Bronchial Secretion Samples

Analysis	Diagnosis	Number	Percentage
FISH	+	31/65	48
FISH	-	28/65	43
FISH	Equivocal diagnosis	6/65	9
Cytology	+	34/66	52
Cytology	-	28/66	42
Cytology	Equivocal diagnosis	4/66	6

The specificity detected by FISH and cytology analysis for the 84 specimens negative for lung cancer (as determined by surgical pathological analysis) is depicted in

Table 21 (49 bronchial brushing samples) and Table 22 (35 bronchial secretion samples). It is expected that among those samples described in Tables 21 and 22 that were negative by surgical pathological analysis, but positive by FISH analysis, there may be some specimens that contain cancerous and/or pre-cancerous cells that were not identified by the surgical pathology methods. In such cases, FISH can allow for an early detection of lung cancer.

Table 21 Specificity of FISH and Cytology for Bronchial Brushing Samples

Analysis	Diagnosis	Number	Percentage
FISH	+	10/49	20
FISH	-	38/49	78
FISH	Equivocal diagnosis	1/49	2
Cytology	+	2/49	4
Cytology	-	47/49	96
Cytology	Equivocal diagnosis	0/49	0

Table 22 Specificity of FISH and Cytology for Bronchial Secretion Samples

Analysis	Diagnosis	Number	Percentage
FISH	+	3/35	8
FISH	-	31/35	88
FISH	Equivocal diagnosis	1/35	3
Cytology	+	4/35	11
Cytology	-	29/35	83
Cytology	Equivocal diagnosis	2/35	6

Other Embodiments

It is to be understood that, while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention. Other aspects, advantages, and modifications of the invention are within the scope of the claims set forth below.

What is claimed is: